Partnership for the Assessment of Risks from Chemicals

Additional Deliverable 4.3

Derivation of HBM guidance values (HBM-GVs) - first set

WP 4 – T4.1

PARC Partnership for the Assessment of Risks from Chemicals

Co-funded by the European Union

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Overall introduction

Within the framework of PARC, WP4, project 4.1.3.1.a, health-based human biomonitoring guidance values (HBM-GVs) were derived for 11 substances, which can be directly compared with HBM data and which indicate the concentration limit in human biological material up to which no harmful effects are to be expected. By definition, the human biomonitoring guidance value for the general population (HBM-GV_{GenPop}) represents the concentration of a substance (or its metabolite(s)) in human biological material (usually urine or blood, rarely other tissues) up to which, according to current knowledge, no adverse health effects are to be expected when considering individual substances. An exceedance of this value should be a reason to investigate the causes and to minimise possible sources of exposure. The HBM-GV derived for occupationally exposed adults (HBM-GV_{Worker}) represents the concentration of a substance or its relevant metabolite(s) in human biological media aiming to protect workers exposed to the respective substance regularly (each work day), and over the course of a working life from the adverse effects related to medium- and long-term exposure. The strategy already developed as part of HBM4EU was used to derive the values (https://doi.org/10.1016/j.ijheh.2020.113622). For one substance, chromium VI, the assessment values refer to selected cancer risk levels for workers in the chrome-plating sector and for welders (HBM Exposure Equivalents for Cancer Risk - HBM-EECR).

With two exceptions, which were made before the prioritisation of substances within PARC was completed, the substances processed are PARC aligned study scope substances. In summary, twelve substance dossiers, including the derivation of HBM-GVs or the HBM-EECR, have have been finalised by project month 36, respectively for DHHB (diethylamino hydroxybenzoyl hexyl benzoate, Uvinul® A plus), benzophenone-3, (gamma/lambda) cyhalothrin, acetamiprid, imidacloprid, diisononyl phthalate (DiNP), diethylhexyl terephthalate (DEHTP), aluminium, nickel, chromium VI, methyl and elemental/inorganic mercury. A scoping document on PFAS was also prepared.

Partners involved in the project P4.1.3.1.a are ANSES, AUTH, LNS, MOH-IL, NIJZ, NIPH, SRU, SECO, TTL, Unisante, UBA, and UOULU. Proposals for assessment values were first discussed within the project and then forwarded to the national hubs for further comments. All partners were involved in discussing the proposals for the assessment value derivations, some of the partners have taken the lead for individual substances and their assessment value derivation (see the following substance-specific documents).

As jointly agreed values under PARC, the HBM-GVs are a valuable instrument for the harmonised assessment of the health risks of individual substances as part of substance regulation, but also in the area of event-driven investigations.

In addition, the substance dossiers compiled here, the assessment values or even just their derivation pathway (if other relevant effect endpoints are to be considered) provide a good basis for an assessment of substance mixtures to which humans are generally exposed, which is required in a next step.

The qualitative assessment of the confidence level for the derived values shows that a higher confidence level could be achieved for some substances if the data situation were improved.

It is planned to publish the main results of this part of the project in a scientific journal in the near future, so that overviews and a comprehensive discussion can be found there.

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Part I

Substance monograph for **DHHB** (trade name: Uvinul® A Plus, CAS-No.: 302776-68-7) and derivation of HBM guidance values (HBM-GVs) for the general population

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Lead Beneficiary/ Responsible AE	UBA
Contributing Participants	Nancy Hopf (Unisanté)/ nancy.hopf@unisante.ch Robert Pasanen-Kase (SECO)/ robert.pasanen-kase@seco.admin.ch Tamar Berman (MOH-IL) / tamar.berman@moh.gov.il
Responsible author(s)	Petra Apel (UBA) / petra.apel@uba.de Jens-Uwe Voss on behalf of UBA Antje Gerofke on behalf of UBA
Internal Reviewers	WP4 co-leaders
External Reviewers from	Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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[1] PU = Public
PP = Restricted to other programme participants (including the Commission Services)
RE = Restricted to a group specified by the consortium (including the Commission Services)
CO = Confidential, only for members of the consortium (including the Commission Services)

Document history

Version	Date	Reviewer	Description of changes
1	2022/2023	Discussion within project, comments by P4.1.3.1.a partners	See Annex of AD 4.3
2	March 2023 till July 2023	Experts nominated by German NH, Swedish NH, UK NH, Austrian NH	See Annex of AD 4.3
3	January 2025	Adoption by P4.1.3.1.a partners	See Annex of AD 4.3, one correction that has been taken into account
4 Final Version	May 2025	Review of AD 4.3 by EAA	Update of 24h bodyweight-related urine volume for adults according German HBM Commission, 2025 (submitted); recalculation of HBM-GV

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Abstract

Under PARC, WP4, project 4.1.3.1.a, human biomonitoring guidance values (HBM-GVs) are derived for priority substances. These HBM-GVs can be directly compared with HBM data and, as jointly agreed values under PARC, are a valuable tool for the harmonised assessment of health risks. The HBM-GVs are derived as needed for the general population and/or occupationally exposed adults.

The human biomonitoring guidance value for the general population ($HBM\text{-}GV_{GenPop}$) represents the concentration of a substance (or metabolite) in human biological material (mostly urine, rarely blood or other tissues) up to which, according to current knowledge - when considering individual substances - there is no risk of adverse health effects. An exceedance of this value should be a reason to investigate the causes and to minimise possible sources of exposure (Apel et al., 2020).

$HBM\text{-}GV_{GenPop}$ have been derived for the UV-filter diethylamino hydroxybenzoyl hexyl benzoate (DHHB, CAS No. 302776-68-7; trade name: Uvinul® A Plus) referring to the two main specific metabolites 2-(4-amino-2-hydroxybenzoyl)benzoic acid (amino-hydroxybenzoyl-benzoic acid) (AHB) and 2-(4-ethylamino-2-hydroxybenzoyl)benzoic acid (ethylamino-hydroxybenzoyl-benzoic acid) (EHB) in urine. DHHB has a high production volume and could be found in the majority of human biomonitoring samples analysed from the German Environmental Specimen Bank (ESB) (sampling years: 2000 to 2021).
The resulting $HBM\text{-}GV_{GenPop\ (children,\ men)}$ are: 3.5 mg/L urine for AHB and 7.9 mg/L urine for EHB, and for the sum of both metabolites 11.4 mg/L urine. The resulting $HBM\text{-}GV_{GenPop\ (women)}$ are 3 mg/L for AHB and 6.8 mg/L for EHB and for the sum of both 9.8 mg/L.[1]

Key Words

Human biomonitoring, DHHB, Uvinul® A Plus, human biomonitoring guidance values, HBM-GV, risk assessment, chemical policy, PARC

[1] By using the outdated 24h bodyweight-related urine volume for men and women of 0.02 L/(kg bw x d) for the calculation of HBM-GV, the resulting $HBM\text{-}GV_{GenPop\ (adults)}$ would have been 5.2 mg/L for AHB and 11.9 mg/L for EHB.

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Authors and Acknowledgements

Authors

Antje Gerofke, UBA, Germany

Petra Apel, UBA, Germany

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We would like to thank the experts who provided us feedback on this work during the consultation period. Comments have been taken into account as far as possible. An overview of the substantive comments and how they were dealt with can be found in the annex to this deliverable comprising various reports.

Background

Within the framework of PARC, HBM guidance values (HBM-GVs) are set for priority substances as needed for the general population (HBM-GV_{GenPop}) and/or for workers (HBM-GV_{worker}) after their derivation approach underwent a consultation process by national experts nominated by PARC partners. The general methodological approach has been published in 2020 (Apel et al., 2020).

As toxicologically derived values referring to the internal exposure biomarker concentration, HBM-GVs can directly be compared with internal exposure levels measured in HBM studies in the general population or at the workplace. Thus, HBM-GVs serve as a useful screening tool for a health-related interpretation of population's exposure levels to identify potential health risks from the exposure to environmental chemicals. Also, HBM-GVs can be used by policy makers to identify possible regulatory needs or to evaluate measures already implemented.

In the current report, the derivation of HBM-GVs for the general population (HBM-GV_{GenPop}), including children and adults, are presented and discussed for the UV-filter diethylamino hydroxybenzoyl hexyl benzoate (DHHB, CAS no. 302776-68-7; trade name: Uvinul® A Plus). DHHB has a high production volume and could be found in the majority of human biomonitoring samples from the German Environmental Specimen Bank (ESB) in the years 2000 to 2021. The detailed metabolism in humans of DHHB and the urinary excretion of the three specific metabolites for monitoring DHHB has been published by Stoeckelhuber et al. (2020)(the excretion fraction after 72 hours is given in the brackets): 2-(4-amino-2-hydroxybenzoyl)benzoic acid (amino-hydroxybenzoyl-benzoic acid) (AHB) (16%), 2-(4-ethylamino-2-hydroxybenzoyl)benzoic acid (ethylamino-hydroxybenzoyl-benzoic acid) (EHB) (33%) and 2-(4-diethylamino-2-hydroxybenzoyl)benzoic acid (diethylamino-hydroxybenzoyl-benzoic acid (DHB) (5%). All 3 metabolites correlate strongly with each other. DHB, however, has the lowest urinary excretion fraction and the highest LOQ, making it less preferable for HBM purposes compared to AHB and EHB. Therefore, HBM-GV_{GenPop} were derived only for AHB and EHB in urine. Additionally, HBM-GVs were given for the sum of both metabolites, in the event that both metabolites were measured in HBM studies.

HBM-GV_{worker} were not derived by the authors of this report, as in Germany the values for the working population are not derived by UBA but by other authorities. Traditionally, the German Environment Agency has focused on the derivation of statistical reference values and health-related HBM values/HBM guidance values for the general population, as these are required for the evaluation of the human biomonitoring studies carried out by UBA. The examination of whether an HBM-GV_{worker} can be derived for DHHB and the corresponding work will be carried out in the extension period of the project P4.1.3.1.a by partners with expertise in the derivation of such values.

Results

Health-based HBM guidance values (HBM-GVs) have been derived for the general population for DHHB for two different urinary DHHB metabolites: AHB and EHB. The resulting HBM-$GV_{GenPop\ (children,\ men)}$ are: 3.5 mg/L urine for AHB and 7.9 mg/L urine for EHB, and for the sum of both metabolites 11.4 mg/L urine. The resulting HBM-$GV_{GenPop\ (women)}$ are 3 mg/L for AHB and 6.8 mg/L for EHB and for the sum of both 9.8 mg/L.

Abbreviations and Acronyms

AF	Assessment Factor
AHB	2-(4-amino-2-hydroxybenzoyl)benzoic acid (amino-hydroxybenzoyl-benzoic acid)
BASF	Badische Anilin und Soda Fabrik
BMUV	Bundesministerium für Umwelt, Naturschutz, nukleare Sicherheit und Verbraucherschutz (Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection)
bw	body weight
CAS	Chemical Abstracts Service (division of the American Chemical Society)
CLP	Classification, Labelling and Packaging of substances
d	day
DHB	2-(4-diethylamino-2-hydroxybenzoyl)benzoic acid (diethylamino-hydroxybenzoyl-benzoic acid,
DHHB	Diethylamino hydroxybenzoyl hexyl benzoate (hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate)
DNEL	Derived No-Effect Level
EC	European Commission
ECHA	European Chemicals Agency
EHB	2-(4-ethylamino-2-hydroxybenzoyl)benzoic acid (ethylamino-hydroxybenzoyl-benzoic acid)
ESB	German Environmental Specimen Bank
ESI	Electrospray Ionisation
F1	First filial generation
F2	Second filial generation
FDA	US Food and Drug Administration
Fue	Urinary excretion factor
GD	Gestational day
GLP	Good Laboratory Practice
GM	Geometric mean
h	hour
HBM4EU	European Human Biomonitoring Initiative
HBM-GV	Human biomonitoring guidance value
kg	kilogramme
LC50	Lethal concentration 50 %
LC/MS	Liquid Chromatography/Mass Spectrometry
LD50	Lethal dose 50 %
LLoQ	Lowest Limit of Quantification
LOAEL	Lowest Observed Adverse Effect Level

Log P_{OW}	Decadic logarithm of the octanol/water partition coefficient
MMAD	Mass Median Aerodynamic Diameter
MoS	Margin of Safety
No	Number
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
OECD	Organisation for Economic Co-operation and Development
P0, P1, P2	Parental generation 0, parental generation 1, parental generation 2
PBPK (PBTK)	Physiologically based pharmacokinetic (toxicokinetic) modelling
PND	Postnatal day
POD	Point of departure
RAC	Risk Assessment Committee
REACH	Registration, Evaluation and Authorisation of Chemicals
RIVM	National Institute for Public Health and the Environment of the Netherlands
SCCS	Scientific Committee on Consumer Safety
SCCP	Scientific Committee on Consumer Products
SCCNFP	Scientific Committee on Consumer Products and Non-food Products
SCOEL	Scientific Committee on Occupational Exposure Limits
SD	Standard deviation
SED	Systemic exposure dose
SVHC	Substance of very high concern
TAF	Total assessment factor
TDI	Tolerable daily intake
TRV	Toxicity (toxicological) reference value – health-based external exposure guidance value proposed by a European or relevant non-European institution (e.g. TDI, RfD, OEL)
TRV-like value	Value for external exposure guidance, calculated according to generally accepted rules, e.g. ECHA 2012 Chapter R.8
UBA	Umweltbundesamt (German Environment Agency)
UV	Ultraviolet
VCI	Verband der Chemischen Industrie
WHO	World Health Organisation

1 Introduction

In the current report, the derivation of HBM-GVs for the general population (HBM-GV_{GenPop}) both for children and adults are presented and discussed for the benzophenone diethylamino hydroxybenzoyl hexyl benzoate (DHHB, CAS no. 302776-68-7, trade name: Uvinul® A Plus).

DHHB absorbs UV-A radiation mainly in the range between 340 to 400 nm (UV-A I), but also at 320 - 340 nm (UV-A II), its absorption maximum is 354 nm (in ethanol) (Durand et al., 2009; Haque et al., 2016). It is mainly used as a UV-filter in sun cream and cosmetic products (face care products with anti-aging effects), but can also be found, for example, in perfumes (BASF, 2013; BASF, 2020; Danish EPA, 2015; Uter et al., 2014). In January 2005 BASF has received the European approval by the EU Commission for Uvinul® A Plus for use of up to 10 % in sunscreen products (http://www.corporate.basf.com/en/investor/strategie/kunden/uvinul.htm).

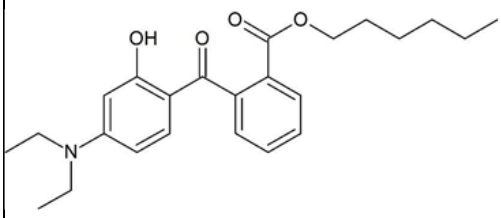
This assessment is based on the data from the registration dossier of DHHB (CAS-No.: 302776-68-7, EC-No.: 443-860-6, full dossier, ≥1,000 t/a) (ECHA Dissemination, 2020; accessed on 06.12.2022; last modified 31.05.2022). Additionally, further evaluations such as the toxicological assessment by the Danish Environmental Protection Agency (Danish EPA) (2015), the Scientific Committee on Consumer Products (SCCP) (2006; 2008), and the Scientific Committee on Cosmetic Products and Non-food Products intended for Consumers (SCCNFP) (2003) were taken into account for this evaluation. Essential information concerning human metabolism and excretion for calculating the HBM-GVs was taken from Stoeckelhuber et al. (2020). The literature review of this report was completed on 6th December 2022 using the PubMed database (without date restrictions), to provide a comprehensive coverage of human Uvinul A Plus exposure sources, levels, and adverse effects in experimental animals and humans. The search was conducted using the terms “Uvinul A Plus” OR DHHB OR “Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate”.

The state of epidemiological knowledge about human exposure to DHHB and whether and how it might affect human health is insufficient to derive HBM-GVs directly from human data. Neither are there any toxicity reference values (TRVs) that are considered suitable for the derivation of HBM-GVs. Thus, the derivation of HBM-GVs for the general population relies on toxicity findings from animal studies (i.e., oral rat studies) with a conversion to human urinary metabolite levels using human data by Stoeckelhuber et al. (2020). Developmental toxic effects of DHHB have been selected as critical effects.

The initial proposal for the derivation of HBM-GVs was based on a dossier by Jens-Uwe Voss on behalf of the German Environment Agency, updated by current literature.

2 General information and physical-chemical properties of DHHB

Table I.1: General information on DHHB

Substance	**Diethylamino hydroxybenzoyl hexyl benzoate (DHHB)**
Trade name	Uvinul® A Plus
IUPAC name*	Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate
INCI name#	Diethylamino hydroxybenzoyl hexyl benzoate
CAS-number*	302776-68-7
EC-number*	443-860-6
Molecular formula*	$C_{24}H_{31}NO_4$
Molecular mass*	397.52 g/mol
Structural formula*	 Figure I.1: Structural formula of DHHB Source: ECHA dissemination, 2020

*: Data taken from the registration dossier (ECHA Dissemination, 2020); # Data taken from the cosmetic ingredient (CosIng) database (CosIng, 2020)

DHHB is an organic substance, which is a white solid powder at room temperature (ECHA Dissemination, 2020). It has a low volatility. As an organic substance with a log Pow of 6.2, DHHB is hardly soluble in water, but soluble in oil (SCCNFP, 2003; SCCP, 2006; SCCP, 2008). Further physical-chemical properties of DHHB are summarized in Table I.2.

Table I.2: Physical-chemical properties of DHHB

Substance	DHHB*
Vapour pressure (at 20 °C)*	2.9×10^{-6} Pa
Melting/ freezing point* (at 1,013 hPa)	54 °C
Boiling point (at 1,013 hPa)*	314 °C (decomposition before boiling)
Relative density (at 20 °C)*	1.156
Log P_{OW} (at 20 °C, pH 6.9)*	6.2
Water solubility (at 20 °C, pH 6.9)*	16 µg/L
Physical state at 20 °C, 1,013 hPa*	Solid
Colour*	White

*: Data taken from the registration dossier (ECHA Dis- semination, 2020); # Data taken from the cosmetic ingredient (CosIng) database (CosIng, 2020)

DHHB is photostabile and does not lose its photoprotective and photostabilising properties after exposure to UV light (Gong et al., 2015; Klimová et al., 2015; Kockler et al., 2013). Gong et al. (2015) investigated the degradation of DHHB in an aqueous solution by UV/hydrogen peroxide (H_2O_2) in steady-state and transient-state photolysis experiments. Neither UV irradiation (λ = 254 nm) nor hydrogen peroxide alone did result in an obvious degradation of DHHB. Under UV irradiation hydrogen peroxide will generate hydroxyl radicals, which degrade DHHB by oxidation following a pseudo-first-order kinetic (Gong et al., 2015).

DHHB has a water solubility of 0.016 mg/L at 20°C, a vapour pressure of 2.9×10-6 Pa at 20°C, and a log Kow of 6.2 (ECHA Dissemination, 2020). The substance is likely to adsorb strongly onto sediment and soil and be relatively immobile in such media. DHHB is not readily biodegradable in the manometric respirometry test (OECD 301F test guideline) and so is potentially persistent (Danish EPA, 2015). Recently, Santos and da Silva (2021) studied the degradation of DHHB whereby considering environmentally-relevant characteristics, such as water pH, chlorine levels, water temperature and dissolved organic matter (DOM) concentration, in order to determine their influence on the degradation reactions. The authors found that DHHB degrades quite rapidly in typical drinking water disinfection conditions. As far as the non-volatile disinfection by-products (DBPs) are concerned, only the mono and dichlorinated forms of DHHB were detected in the degradation reactions. Regarding influential variables on DHHB degradation, the presence or absence of DOM in solution did not alter the trends that were found (degradation of DHHB is more significant at lower pH values and higher levels of active chlorine in solution). In the photo-degradation studies, DHHB was found to be extremely photo-stable, with only about 15% degradation rate detected during artificial irradiation periods of 6 h (Santos and da Silva, 2021).

3 Occurrence and use of DHHB

DHHB is an anthropogenic substance without natural occurrence. In the European Economic Area ≥ 1,000 tons of DHHB are manufactured and/or imported per year (ECHA Dissemination, 2020). According to the registration dossier, DHHB is only used in cosmetics and personal care products in the EU. Thus, predominantly consumers and professional workers come into contact with DHHB, the latter during manufacturing, formulation or repackaging (ECHA Dissemination, 2020).

The occurrence of DHHB in different cosmetic products was assessed in a German survey from 2006 to 2009 which investigated a total of 4,447 products, which were categorised according to Annex I of the Cosmetic Directive (76/768/EEC). Only 1.8% of these 4,447 products contained DHHB according to information on the label. Surprisingly, it was most common in perfumes (in 4.8% of the perfume samples; perfumes n=42), followed by sunscreens (in 2.6% of the sunscreen samples; sunscreens n=462), hair products (in 1.9% of the hair product samples, hair products n=54) and lip products (in 1.6% of the lip products, lip products n=62). The product categories cream, make-up, and nail did not contain DHHB (Uter et al., 2014).

In an assessment conducted 2010 in the United Kingdom, DHHB was identified in 5% of the total 337 sunscreens compared to just 1% in 2005 (n=308 sunscreen products) (Kerr, 2011).

The Danish EPA (2015) conducted a shop survey at cosmetic retailers in Denmark and investigated which UV filters or UV absorbers were found in the 291 individual cosmetic products collected during June/July 2014. DHHB was found in 53 of the 291 investigated products. As expected DHHB was mainly present in sunscreens (46 sunscreens from n=126), followed by face creams (3 face creams from n=31), Eau de toilettes (2 Eau de toilettes from n=13), and foundations (2 foundations from n=29).

According to the manufacturers, DHHB is highly compatible with commonly used sunscreens and can be easily combined with other organic and inorganic UV filters to produce sunscreens and other cosmetic products (BASF, 2013).

4 Legal framework

According to the Regulation (EC) No 1223/2009 on cosmetic products, DHHB is permitted up to 10% in sunscreen products in the European Union (EU, 2009). No harmonised classification according to Regulation (EC) No. 1272/2008 is available. No classification is proposed in the registration dossier[2], while in the C&L inventory DHHB is classified as hazardous to the aquatic environment (aquatic chronic category 4, H413, may cause long lasting harmful effects to aquatic life) (ECHA C&L Inventory, 2020).

The available information shows that the substance is unlikely to meet the REACH Annex XIII criteria for either PBT or vPvB (Danish EPA, 2015).

In Japan and Australia, DHHB is also allowed up to a maximum concentration of 10% for cosmetic use (Australian Government, 2020; BASF, 2013). In the United States of America, DHHB is currently not approved for use in sunscreens. However, the registrant is intending a registration (BASF, 2013).

[2] https://echa.europa.eu/registration-dossier/-/registered-dossier/29413/2/1

5 Exposure of the general population

The release to the environment of DHHB is likely to occur from intended use of cosmetic products (ECHA Dissemination, 2020), however, unintended environmental exposure from manufacturing and subsequent downstream activities cannot be excluded.

In seawater samples from six beaches of Gran Canaria, DHHB was detected in 60% of the analysed samples above its detection limit of 1.3 ng/L. The maximum DHHB concentration of 228.7 ng/L was the lowest maximum concentration of all UV filters investigated (e.g. homosalate (536.2 ng/L), octocrylene (1,324.9 ng/L), benzophenone-3 (3,316.7 ng/L)) (Sanchez Rodriguez et al., 2015).

Vila et al. (2016) analysed twelve water samples from Spanish bathing areas (one aquapark, seven swimming pools, two rivers, one spa, and one sea) with respect to their UV filter concentrations (14 UV filters investigated). DHHB was one of the 4 UV filters, which could not be detected in any of the water samples (LoD = 6.0 ng/L).

Due to the use of DHHB in sunscreen products and other cosmetics, skin exposure is considered to be the main route of exposure for the general population. But also hand to mouth contact has to be considered as well as inhalation if spray products are used. Exposure of professional workers occurs during manufacturing, formulation and repacking. Information on occupational exposures can be found in the Annex (taken from the registration dossier).

No population representative human biomonitoring studies are available for DHHB. However, data are available for DHHB metabolites AHB and EHB (for chemical structures see chapter 8) from the German Environmental Specimen Bank (ESB) for young adults (aged 20-30 years) for the years 2000, 2008, 2012, 2016 and 2021 (Table l.3, preliminary information, publication now available: https://doi.org/10.1016/j.ijheh.2025.114565). This data shows that urinary metabolites of DHHB can be found in the majority of samples analysed. Also, Stoeckelhuber et al. (2019) showed that after dermal application of a sunscreen product urinary DHHB excretion was still quantifiable 72 hours after the application.

In connection with their method validation, Stoeckelhuber et al. (2019) published data on 58 German volunteers, quantifying AHB in 32.8% and EHB in 34.5% of the samples.

Table I.3: AHB and EHB measured in samples from the German ESB (2000-2021) (preliminary information)

Metabolite	Group size	% > LoQ	GM µg/L	Maximum µg/L
AHB	60 per year (2000 – 2021)	76-94	0.2-0.4	1.4-53
EHB	60 per year (2000 – 2021)	0-38	n.d – 0.12	n.d.-89

6 Toxicological profile of DHHB

6.1 Toxicokinetics of DHHB

6.1.1 Human data

Five volunteers (3 males and 2 females, aged between 28 and 68) were once orally exposed to 0.295 mg DHHB/kg bw in water/ethanol (100:1 v/v) and urine samples were collected before and up to 72 hours after dosing (Stoeckelhuber et al., 2020). 24 and 48 hours after administration about 85% and 95%, respectively, of the administered dose was excreted in the urine in the form of all detectable metabolites. After 72 h, excretion was still not fully completed. After 72 hours, 54% of the applied dose were recovered in urine in form of three main metabolites. On a molar basis average, EHB, AHB, and DHB amounted to 32.8%, 16.3% and 5.1% of the doses administered, respectively (see table I.4).

DHHB was not detected in any urine sample. In urine, the maximum concentrations of the three metabolites EHB, AHB, and DHB were measured two hours after dosing. DHB mostly occurred in form of glucuronides (90%), while only 63% of EHB and 31% of AHB were conjugated to glucuronic acid. The elimination half-lives of all three metabolites were about 4.5 hours. Additionally, single hydroxylated species of the 3 main metabolites were detected in urine but estimated to be present at a 100-fold lower concentration than the main metabolites.

Table I.4: Urinary excretion factors (Fue) for DHHB metabolites (Stoeckelhuber et al., 2020)

	AHB	EHB	DHB
Urinary excretion factor (72 h)	0.163	0.328	(0.051)

Based on the urinary excretion of DHHB metabolites in a study with 58 volunteers, the mean daily exposure to DHHB was estimated. By applying the conversion factors (ratio between the total dose administered and the amount of metabolite (or sum of several metabolites) to urinary metabolite concentrations, they calculated very similar mean oral equivalent DHHB doses of 9.3 µg/d based on AHB and 9.2 µg/d based on EHB. If the calculation of the equivalent oral dose was based on the conversion factor for DHB (4.5 µg/d) a clear discrepancy from the other calculated oral doses existed, which might be caused by its much lower urinary excretion fraction resulting in low measured concentrations or results below the LoQ, which lead to a high uncertainty (Stoeckelhuber et al., 2020).

Furthermore, the same five volunteers who had been orally exposed (see above) were skin exposed to a commercial sunscreen formulation containing 10% DHHB (59 mg/kg bw, total amount 3.66 - 5.31 g Uvinul® A Plus). The skin application was performed by spiking a commercially available Uvinul A plus®-free sunscreen with the test compound to a total amount of 10% (w/w). A defined amount of 0.59 g/kg body weight of the spiked sunscreen was applied to the arms, neck and chest of the subjects. Subjects were asked not to wash the relevant body parts until the cream was completely absorbed into the skin (approx. 15–30 min).

The three main metabolites (AHB, EHB, and DHB) were detected in urine after 3 hours and an explicit increase was observed after 24 hours. DHHB was not detected in any sample. Excretion of all metabolites occurred in waves with observed maxima at 24, 43, and 56 hours, but still not being completed after 72 hours.

Resorption rates of 0.0034 - 0.0214% of the applied dermal dose were calculated based on the cumulated concentrations of AHB, EHB, and DHB (Stoeckelhuber et al., 2020).

In an *in vitro* study on percutaneous absorption (similar to OECD TG 428, GLP-compliant) using human skin and Franz diffusion cells (abdominal skin, 3 female donors) a dermal absorption of 0.54% was determined after 24 hours exposure (ECHA Dissemination, 2020). 18 mg of a test formulation containing 10% radio-labelled Uvinul® A Plus (purity: 99.5%, mean applied dose: 1.8 mg) were applied to the skin area in the diffusion cell (approximately 10 mg/cm^2 skin) for 24 h. The test formulation was then washed off. The skin wash contained 96.95% to 103.73% of applied radioactivity. In the receptor fluid 0.02 ± 0.01% of radio-labelled Uvinul® A Plus were detected. The mean recovery of radioactivity in the stratum corneum (tape stripping, without stripes 1 and 2) was 0.41 ± 0.30%.

Lesser amounts of radio- activity (0.11 ± 0.05%) were found in the remaining skin after tape stripping. The mean recovery rate was 101.08 ± 10.06% (ECHA Dissemination, 2020).

A further *in vitro* study with Franz-type diffusion cells investigated the permeation of 3.5% DHHB in an oil in water emulsion through human skin (abdominal skin, female donors aged 25 - 60). The applied amount was 2 mg/cm^2 on a diffusion area of 5.31 cm^2. After 24 hours no penetration of DHHB through human skin was observed. The authors of the study regarded DHHB's high molecular weight, high log Pow, and the low applied concentration as the reasons for not observing any skin permeation (Durand et al., 2009). But this is clearly opposite to human dermal application study of Stoeckelhuber et al. (2020), which showed measurable uptake of DHHB.

6.1.2 Animal data

Bioavailability after oral application was assessed in rats which received a single dose of radio-labelled Uvinul® A Plus (dose: 100 mg/kg bw, purity: 99.5%, vehicle: olive oil). Bioavailability was calculated by summing up the radioactivity found in bile, urine, cage wash, and carcass within 72 hours. Total bioavailability was 26% and 45% of administered dose in males and females, respectively. The measured radioactivity in residual carcass of males and females was 0.46% and 1.26%, respectively, the distribution of DHHB was not determined. Most of the orally applied dose was excreted within 72 hours with faeces (51.47% males and 32.78% females), urine (13.79% males and 22.62% females), bile (11.28% males and 19.76% females), and only a minor amount was found in the cage wash (0.71% males and 1.46% females) (ECHA Dissemination, 2020).

A toxicokinetic study in rats (similar to OECD TG 417, GLP-compliant) was performed with crystalline and molten Uvinul® A Plus, which was administered once orally in a low (1,000 ppm) or high dose (10,000 ppm) via diet (ECHA Dissemination, 2020). Due to uncertainties regarding the sampling times, the dosage as well as the duration of feed intake, this study was not considered further.

In an *in-vivo* percutaneous absorption study (according to OECD TG 427 and GLP) male Wistar rats were exposed to a formulation containing 10% radio-labelled Uvinul® A Plus (purity: >95%, 0.81 mg/cm^2, approx. 2.6 mg/kg bw, specific activity: 6.78% MBq/mg) on approx. 10 cm^2 for 12 hours. The total absorbed amount of radioactivity in excreta, cage wash, carcass, blood cells and plasma after 12, 24, and 120 hours were 2.96%, 2.31%, and 3.16%, respectively. The mean total recovery of radio-activity was between 104.99% and 116.88% of the applied total radioactivity (ECHA Dissemination, 2020).

The dermal absorption of Uvinul® A Plus (purity: 99.5%, mean dose applied: 1,777.25 µg, radioactively labelled) in female rats was determined in an in vitro study on percutaneous absorption (similar to OECD TG 428, GLP-compliant). Due to shortcomings in the quality of this study, this study was not considered further.

In a Franz diffusion cell-system the *in-vitro*-penetration of a cosmetic formulation, which contained 10% radio-labelled DHHB in an oil/water emulsion and was applied to skin samples of three female pigs (thickness 500 µm, dose: 2 mg/cm^2, 200 µg active incredient/cm^2 for 24 hours), was measured. At the end of the exposure, no DHHB was detected in the receptor fluid. In the upper layer of stratum corneum a mean dose of 0.77% was absorbed and in the deeper skin layers of 0.042%. The mean recovery was 93%. According to SCCP, the mean dermal absorption was 0.100 ± 0.115 µg/cm^2 or 0.042 ± 0.050% of mean dose with a maximum value of 0.310 µg/cm^2 and 0.149% (ECHA Dissemination, 2020; SCCP, 2006; SCCP, 2008).

Further *in vitro* studies performed with porcine skin in Franz diffusion cell-systems did not detect DHHB in the receptor fluid, but detected it in stratum corneum, epidermis, and dermis (Haque et al., 2016; Souza and Maia Campos, 2017).

6.2 Toxicity of DHHB

6.2.1 Acute toxicity

Acute oral toxicity of Uvinul® A Plus is low for humans and rats. After a single oral exposure to Uvinul® A Plus (0.295 mg/kg bw diluted in ethanol/water) no signs of toxicity were observed in humans (ECHA Dissemination, 2020). LD50 values for rats (OECD TG 423 and GLP) were higher than 2,000 mg/kg bw (gavage, vehicle: olive oil or 5% Tylose in aqua dest.). No toxicity was observed in dosed animals (ECHA Dissemination, 2020; SCCNFP, 2003; SCCP, 2006; SCCP, 2008).

In an acute inhalation toxicity study (OECD TG 403 and GLP) Wistar rats were once nose-only exposed to 5.22 mg/L Uvinul® A Plus dust (purity: 99.7 area%, mass median aerodynamic diameter (MMAD): 2.52 µm) for 4 hours. Except irregular respiration of all rats until day 2, no further adverse toxicity signs were observed (LC0: 5.22 mg/L air (analytical)) (ECHA Dissemination, 2020).

There are no data on acute toxicity via the dermal route available (ECHA Dissemination, 2020).

6.2.2 Irritation and sensitization

In two eye irritation studies (according to OECD TG 405 and GLP), undiluted Uvinul® A Plus led to slight conjunctival redness in single rabbits, which was fully reversible within 48 or 72 hours and did not require classification for effects on the eyes (ECHA Dissemination, 2020; SCCNFP, 2003; SCCP, 2006; SCCP, 2008).

In rabbits (n=3) the semi-occlusive application (OECD TG 404 and GLP) of 0.5 ml undiluted Uvinul® A Plus (purity: 97.9%) for 4 hours resulted in moderate to severe erythema (grade 2 - 3) in all animals one hour after removal of the patch. After 48 and 72 hours, erythema were eased to slight (grade 1) and were reversible within 7 days. The test substance was classified as not skin irritating (ECHA Dissemination, 2020). Another acute dermal irritation study in rabbits exposed to 5 g Uvinul® A Plus confirmed the observed dermal reaction and classification (ECHA Dissemination, 2020; SCCNFP, 2003; SCCP, 2006; SCCP, 2008).

No data or studies are available regarding respiratory tract sensitization of DHHB.

In a guinea pig maximisation test, according to OECD TG 406, DHHB (purity: 99.35 g/100 g, intradermal induction: 5% in olive oil; occlusive epicutaneous induction: 25% in olive oil; challenge: 25% in olive oil, epicutaneous occlusive) did not induce skin sensitization in female guinea pigs (control n=5, test group: n=10) (ECHA Dissemination, 2020; SCCNFP, 2003; SCCP, 2006; SCCP, 2008). The registrant rated the study as reliable without restriction (RL 1). However, SCCNFP/SCCP regard this study as invalid without mentioning any reasons (SCCNFP, 2003; SCCP, 2006; SCCP, 2008). OECD guideline 406 requires the use of at least 10 laboratory animals and 5 control animals. The guideline also recommends that in case of a negative result, the number of test animals should be increased to 20 and the number of control animals to 10, which was not taken into account in this study according to the data provided by ECHA Dissemination (2020).

In a photo-patch test (as a double-blind study) with 80 volunteers, no irritant effects were found at test concentrations of 2, 5 and 10% DHHB in petrolatum (Kerr et al., 2009). This test was performed to gain knowledge on the maximum concentration of organic sunscreen filters in current usage that does not lead to irritant reactions when performing photopatch testing, since such irritant reactions can be misinterpreted as positive photoallergic contact dermatitis reactions (Kerr et al., 2009).

Case reports and human patch tests indicate that DHHB could act as a contact allergen and photo-contact allergen. Out of 1,031 patients four (i.e., 0.38%) had positive photo-patch and three (i.e., 0.29%) had positive patch reactions in the European multicenter photo-patch test study (Kerr et al., 2012).

A phototoxicity study with mouse Balb/c 3T3 cells clone 31 (similar to OECD TG 432, GLP-compliant) exposed to eight concentrations of DHHB ranging from 0 to 100 µg/mL either in the dark or after UV irradiation (> 320 nm, dose: 1.7 mW/cm^2, for 50 min at room temperature (= 5 J/cm^2)) did not show a significant difference in cell survival between the exposed and unexposed cells (SCCNFP, 2003; SCCP, 2006; SCCP, 2008).

A phototoxic or photoallergenic potential of DHHB (10 or 20% diluted in olive oil, 0.2 mL) was not observed in guinea pigs exposed to UV-A (365 nm, 9 J/cm^2) or UV-B radiation (312 nm, 0.1 J/cm^2) (SCCNFP, 2003; SCCP, 2006; SCCP, 2008).

6.2.3 Repeated dose toxicity

6.2.3.1 Human data

For humans, no data on repeated dose toxicity of DHHB is available.

6.2.3.2 Animal data

In animals, repeated dose toxicity data are only available for the oral route for rats.

Wistar rats (n=10 per dose group and sex) were orally exposed to 0, 600, 3,000 or 15,000 ppm DHHB (purity: 99.35%, test substance form: crystalline) in diet for 90 days (according to OECD TG 408 and GLP). The mean daily compound intake based on food intake was 0, 51.7, 250.2, and 1,248.8 mg/(kg bw x d) for males and 0, 59.3, 288.0, and 1,452.1 mg/(kg bw x d) for females. No treatment-related mortality occurred. One male of the high dose group was sacrificed due to a rapidly increasing palpable mass in the abdomen, red-coloured urine (day 28 onwards), piloerection (day 42 onwards), and moribund state on day 49. According to the information in the registration dossier, kidney lesions, which occur spontaneously, are predominantly responsible for the animal's bad condition. Observed incidental findings included: missing vaginal opening and a palpable mass ("scrotum-like") under skin in one female of the control group from day 63 onwards, red-coloured urine and urine smear at anogenital region in one low-dose male from day 77 onwards, exophthalmia in one mid dose female from day 77 onwards, and a tooth anomaly in one high dose female from day 28 onwards. No significant changes were observed in body weights or body weight gain. However, terminal body weights were slightly but not significantly decreased in males (-3.6%) and females (-2.5%). A significant increase in food efficiency on day 84 in females in the medium and high dose groups is considered an incidental finding in the registration dossier. Ophthalmoscopic examination, haematology, clinical chemistry and urinalysis revealed no treatment-related effects in animals of the control and dose groups. During neurobehavioural examination, single incidences or findings equally distributed in animals of treated and control groups occurred in open field observations, sensorimotor, and reflex tests. Males of the mid and high dose group showed significantly increased rearing. This effect was not seen in females and did not affect the motor activity, thus it was considered incidental in the registration dossier.

Females of the low dose group had a significantly increased relative heart weight (+15%) compared to controls. In females of the mid dose group, absolute (-34%) and relative (-31%) spleen weights were significantly decreased compared to controls. In males of the high dose group the relative weight of testes was significantly increased (+9%) compared to controls. The relative weight of liver was significantly increased in males (+7%) and females (+10%) of the high dose group compared to controls. During gross pathology, most observed lesions were identified in the sacrificed high dose male animals and were not treatment-related (dark red coloured lungs, severely enlarged kidneys with granular surface, slight dilation of ureters and moderately decreased organ sizes of seminal vesicles and prostate gland). Two females of the low dose group had uterine dilation.

Otherwise the following single, sporadic, non-treatment related findings were noted in dose groups: erosion/ulcer and hyperemia in glandular stomach, cyst in liver and kidney, dilation of uterus and vagina, reduced size of thymus, enlarged iliac lymph nodes, and sparse hair on skin. Histopathology revealed no treatment-related microscopic findings in any of the investigated organs, including heart, spleen, liver, and testes. The recorded microscopic findings were either single observations, of low incidence, occurred only in controls, or were comparable between control and high dose animals. No treatment-related findings were noted in the sperm analysis. The observed changes in organ weights were not regarded as treatment-related because either absolute weights were not significantly changed, or no gross lesions or microscopic findings were seen, or blood chemistry parameters (liver enzymes) were not changed. Therefore, a NOAEL of 15,000 ppm (1,248.8 mg/(kg bw x d) in males or 1,452.1 mg/(kg bw x d) in females) was derived by the registrant (ECHA Dissemination, 2020). In their evaluations, SCCP or SCCNFP derived a NOEL of 3,000 ppm (250.2 mg/(kg bw x d) in males or 288.0 mg/(kg bw x d) in females) based on the observed relative liver weight increase (without a histopathological correlate) in the high dose group (SCCNFP, 2003; SCCP, 2006; SCCP, 2008). No further details were provided by ECHA Dissemination (2020).

6.2.4 Genotoxicity

In vitro

DHHB was not mutagenic in a gene mutation study (according OECD TG 471 and GLP) in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538, and *Escherichia coli* WP2 uvr up to a maximum test

concentration of 5,000 µg/plate in plate incorporation test and up to 2,500 µg/plate in a preincubation test with and without metabolic activation (ECHA Dissemination, 2020; SCCNFP, 2003; SCCP, 2006; SCCP, 2008). In a gene mutation test with mouse lymphoma L5178Y cells (according OECD TG 473 and GLP) an increase of mutation frequency at the thymidine kinase locus was not observed up to cytotoxic concentrations with and without metabolic activation (ECHA Dissemination, 2020; SCCNFP, 2003; SCCP, 2006; SCCP, 2008). Additionally, no clastogenic activity was induced by DHHB in a chromosome aberration test in Chinese hamster lung fibroblasts V79 cells (according OECD TG 476 and GLP) with and without metabolic activation (ECHA Dissemination, 2020; SCCNFP, 2003; SCCP, 2006; SCCP, 2008).

In a photomutagenicity test with *Salmonella typhimurium* TA 98, TA 100, TA 102, TA 1535 and *Escherichia coli* WP2 (according OECD 471 and GLP) DHHB did not induce gene mutations in test strains up to a maximum concentration of 5,000 µg/plate either in the plate incorporation or preincubation test under irradiation with artificial sunlight (SCCNFP, 2003; SCCP, 2006; SCCP, 2008).

Up to a maximum concentration of 80 µg/mL, DHHB was not clastogenic in a photomutagenicity test (according OECD 473 and GLP) with Chinese hamster V79 cells when exposed to UV-A and UV-B radiation (SCCNFP, 2003; SCCP, 2006; SCCP, 2008).

In vivo

An *in vivo* micronucleus test according to OECD TG 474 and GLP (Uvinul® A Plus: 0, 500, 1,000, and 2,000 mg/kg bw in dimethyl sulfoxide (DMSO), two i.p. applications, purity: 97.9%) conducted in mice (5 animals per sex and dose group) did not show genotoxic activity in the form of chromosome breaks or an increase in micronuclei by DHHB in bone marrow cells (ECHA Dissemination, 2020).

6.2.5 Carcinogenicity

There is no study investigating the carcinogenicity of DHHB available.

6.2.6 Reproductive toxicity

In a two-generation reproduction toxicity study from 2006 (according to OECD TG 416 and GLP) Wistar rats (n=25 per sex and dose in F0 generation and in F1 generation) were exposed daily to 0, 100, 300, or 1,000 mg/(kg bw x d) Uvinul® A Plus in diet (purity: >98.8%, diet preparation: melted at 70 °C, preparation of differently concentrated solutions in acetone, which were sprayed onto small amounts of feed, heated to 40 °C, and mixed with feed until the desired concentrations were reached). Parental males of F0 and F1 generation were exposed for approx. 4 months and females of F0 and F1 generation approx. a week longer. No mortalities occurred in the P0 or P1 parental generations. At 1,000 mg/(kg bw x d) several P0 animals and P1 females showed signs of impaired general state. Mean body weight and body weight gains were statistically significantly decreased in P0 males from 300 mg/(kg bw x d) onwards (about -5% at week 17 and about -7% for weeks 0-17) and in P1 males from the lowest dose 100 mg/(kg bw x d) onwards (about -6% at week 18 and about -6% for weeks 0-18), see Table I.5 for further details. For P0 and P1 females body weights and body weight gains were statistically significantly decreased during various study phases in the highest dose group. Food consumption was statistically significantly reduced for P0 animals and P1 females during various study phases in the highest dose group. An exception were P1 males, which had a statistically significantly reduced food consumption at 100 mg/(kg bw x d) and onwards (see Table I.5). Substance intake was especially impaired in P1 females during gestation at 1,000 mg/(kg bw x d) and during lactation (see Table I.6).

Organ weights, gross pathology or histopathology of parental P0 animals revealed no treatment-related findings. In P1 males increased absolute and relative kidney weights were seen at 1,000 mg/(kg bw x d). Microscopic examination revealed slightly higher amounts of eosinophilic droplets in the tubular epithelial cells, which were identified as alpha2µ-globuline, from 300 mg/(kg bw x d) onwards. No differences between treated P0/P1 or control animals were seen in estrous cycle length and sperm parameters. Reproductive performance was significantly altered in P0 animals in the high dose group: three P0 dams without delivery of pups instead dead implants in utero, decreased gestation index, statistically significantly increased post-implantation loss, and also noted in P1 females statistically significantly reduced mean number of implantation sites and delivered pups per dam (see Table I.5).

Table I.5: Statistically significant effects observed in P0 and P1 animals compared to controls after exposure to Uvinul® A Plus in a 2-generation reproduction toxicity study (ECHA Dissemination, 2020)

	Uvinul® A Plus (mg/(kg bw x d))		
Dose group	**100**	**300**	**1,000**
P0 male			
mean body weight	-	≈ -5% (week 17)	-14% (final weight)
body weight gain	-	≈ -7% (weeks 0-17)	≈ -22% (weeks 0-17)
P0 female			
mean body weight premating gestation lactation	-	-	-11% (week 10) -24% (day 20 p.c.) -10% (day 21 p.p.)
body weight gain premating gestation	-	-	-19% (weeks 0-10) -51% (weeks 0-20 p.c.)
delivery of no pups, but dead implants *in utero*	-	-	3 dams
gestation index	-		83% (100% in control)
post-implantation loss	-		22.1% (4.4% in control)
mean no. of implantation sites per dam	-		9.6 (11.6 in control)
mean no. of delivered pups per dam	-		8.8 (11.0 in control)
P0 male and female			
food consumption	-	-	-11 - -28%
P1 male			
mean body weight*	≈ -6% (final weight)	≈ -8% (final weight)	-24% (final weight)
body weight gain*	≈ -6% (weeks 0-18)	≈ -8% (weeks 0-18)	≈ -25% (weeks 0-18)
food consumption*	≈ -5% (weeks 0-18)	≈ -4% (weeks 0-18)	-15 - -27%
absolute/relative kidney weights	-	-	+16.7%/+64.3%

	Uvinul® A Plus (mg/(kg bw x d))		
Dose group	**100**	**300**	**1,000**
P1 female			
mean body weight premating gestation lactation	-	-	-16% (week 10) -25% (day 20 p.c.) -17% (day 21 p.p.)
body weight gain premating gestation	-	-	-13% (weeks 0-10) -40% (weeks 0-20 p.c.)
mean no. of implantation sites per dam	-		9.4 (10.8 in control)#
mean no. of delivered pups per dam	-		8.8 (10.1 in control)#
P1 male and female			
food consumption	-	-	-15 - -27%

Data taken from the registration dossier. Level of statistical significance was not specified in the registration dossier; # not statistically significantly different compared to controls

* Borderline reduction of food consumption and body weight in one paternal generation only (ECHA dissemination, 2020)

At 1,000 mg/(kg bw x d) mean number of delivered F1 pups and postnatal viability of F1 and F2 pups was decreased (see Table I.7). Lower body weights and body weight gains were seen in F2 pups from 300 mg/(kg bw x d) onwards and in F1 pups at 1,000 mg/(kg bw x d). Secondarily to the general delayed development occurred a slightly delayed sexual maturation of F1 pups at 1,000 mg/(kg bw x d). Additionally, organ weights (brain, thymus, and spleen) of F1 and F2 pups were affected, most pronounced at 1,000 mg/(kg bw x d) (see Table I.7). In the registration dossier a NOAEL for general toxicity for P0 and P1(only female) animals of 100 mg/(kg bw x d) was derived based on observed clinical signs, reduced body weights and food consumptions. For male P1 animals, a LOEL of 100 mg/(kg bw x d) was established due to the slight reduction in body weight accompanied by a slight reduction in food consumption. The effects on food consumption/body weight data were interpreted as indicative of limited palatability of the treated diet and not as specific toxicity per se.

For fertility and reproductive performance a NOAEL of 300 mg/(kg bw x d) and for developmental toxicity for F1 and F2 offspring a NOAEL of 100 mg/(kg bw x d) was derived (ECHA Dissemination, 2020). This study report (from 2006) was not available to SCCP and could therefore not be taken into account in its evaluation from 2008 (SCCP, 2008).

Table I.6: Uvinul® A Plus intake of P0 and P1 animals in a 2-generation reproduction toxicity study (ECHA Dissemination, 2020)

	Uvinul® A Plus (mg/(kg bw x d))		
Dose group	**100**	**300**	**1,000**
P0			
Male	101.3	303.8	1,010.3
female premating	103.7	311.4	1,033.0
female gestation	103.4	312.0	927.8
female lactation	98.7	294.0	952.4
P1			
Male	101.1	303.2	1,009.9
female premating	102.0	305.5	1,017.1
female gestation	101.2	301.7	955.2
female lactation	93.6	281.1	902.5

Table I.7: Statistically significant effects observed in F1 and F2 pups compared to controls after exposure to Uvinul® A Plus in a 2-generation reproduction toxicity study (ECHA Dissemination, 2020)

	Uvinul® A Plus (mg/(kg bw x d))		
Dose group	**100**	**300**	**1,000**
F1 male			
mean absolute/relative spleen weights	-	-	decreased§
F1 female			
mean relative thymus weights	-	increased§	-
mean absolute/ relative spleen weights	decreased§	-	-
F1 male and female			
Viability	-	-	95% (99% in controls)
mean body weight	-	-	-24% (weaning)
body weight gain	-	-	≈ -25% (PND 4-21)
mean absolute/ relative spleen weights	-	- /-7%	-45%/-28%
mean absolute/ relative brain weights	-	-	-6%/+23%
mean absolute/ relative thymus weights	-	-	-25%/ -
F2 female			

	Uvinul® A Plus (mg/(kg bw x d))		
Dose group	**100**	**300**	**1,000**
mean absolute/ relative spleen weights	-	decreased§	-
F2 male and female			
Viability	-	-	85% (99% in controls)
lactation index	-	-	97% (100% in controls)
mean body weight	-	≈ -6% (day 21 p.p.)	-26% (weaning)
body weight gain	-	≈ -7% (PND 4-21)	≈ -28% (PND 4-21)
mean absolute/ relative spleen weights	-	-	-46%/-28%
mean absolute/ relative brain weights	-	-	-7%/+23%
mean absolute/ relative thymus weights	-	-	-24%/ -

Data taken from the registration dossier; level of statistical significance was not specified in the registration; § numerical value not given in the registration dossier

In a subchronic oral study in rats (see section 6.2.3.2 for further details) assessment of sperm parameters, as well as the investigated primary and secondary sex organs (e.g. testis, ovaries, oviducts, uterus, vagina, seminal vesicles, pituitary gland, adrenal glands, prostate gland, and mammary gland) did not show any treatment-related findings (ECHA Dissemination, 2020).

In a prenatal developmental toxicity study from 2001 (according to OECD TG 414 and GLP) Wistar rats (n=25 mated females per dose group) were daily exposed to 0, 40, 200 or 1,000 mg DHHB/(kg bw x d) via gavage (purity: 99.35%, test substance form: crystalline, vehicle: olive oil, 5 mL/kg bw/d) from gestation day 6 to 19. From 200 mg/(kg bw x d) and onwards transient salivation after gavaging occurred and a statistically significantly reduced food consumption was observed (about -7% compared to control for days 6 - 19 p.c. at 1,000 mg/(kg bw x d)). Four females of the highest dose group showed urine-smeared fur from day 9 p.c. onwards. Also, at 1,000 mg/(kg bw x d) occurred a slight reduction in body weight gain (about -8% compared to control for day 6-19 p.c.) and - considering the uterus weight - corrected body weight gain (about -11% compared to control). No treatment-related effects on gestational parameters or fetuses (e.g. embryotoxic or teratogenic effects) were noted up to the maximum dose of 1,000 mg/(kg bw x d). The derived NOAELs were 200 mg/(kg bw x d) for maternal toxicity and 1,000 mg/(kg bw x d) for developmental toxicity (ECHA Dissemination, 2020; SCCNFP, 2003; SCCP, 2006; SCCP, 2008).

6.2.7 Mechanistic findings

DHHB induced slowly rising calcium influxes in human sperm cells at 10 µM, but not in a progesterone-like manner via CatSper channel. After 20 hours of incubation, DHHB did not affect the sperm viability compared to the negative control DMSO. The acrosome reaction or penetration of sperm into methylcellulose were not significantly influenced by DHHB (Rehfeld et al., 2016; Rehfeld et al., 2018).

An increase in uterus weight or change in morphology (histopathology) was not noted in an uterotrophic assay in female Wistar rats (n=10 per dose) after three oral applications of 0, 250, or 1,000 mg DHHB/(kg bw x d) (purity: > 99% by GC, vehicle: olive oil, gavage 5 mL/(kg bw x d)). At 1,000 mg/(kg bw x d) a statistically significantly decreased body weight gain between day 0 and 1 and the mean (no further information given) between day 0 and day 3 (about -24% compared to control) was observed. This effect was regarded as systemic toxicity. In the described test DHHB showed no estrogenic activity (ECHA Dissemination, 2020).

In a Hershberger assay, Uvinul® A Plus (0, 200, or 1,000 mg/(kg bw x d), purity: 97.9%, vehicle: corn oil, gavage 5 mL/(kg bw x d)) was administered orally to castrated male Wistar rats (n=6 per dose) daily for 10 days. Rats, which were treated with the highest dose and testosterone propionate, did not have increased concentrations of testosterone, dihydrotestosterone, and luteinizing hormone in relation to animals which only received testosterone propionate. A dose-response related significant increase in relative liver weight was observed. Examinations (weight and histology) of sexual organs did not identify a difference between treated and control animals. Thus, Uvinul® A Plus was not regarded as having androgenic or anti-androgenic activity (ECHA Dissemination, 2020).

Kunz and Fent (2006) observed no estrogenic activity of DHHB in a yeast-recombinant expressing the rainbow trout estrogen receptor α (rtERα) in concentrations up to 0.25 M DHHB.

7 Evaluation of DHHB by various bodies and conclusion

Derived no effect level (DNEL) values as provided in the registration dossier (ECHA Dissemination, 2020) are summarised in Table I.8. No DNELs were derived for the general population and instead reference was made to the regulation of the substance under the framework of the Cosmetics Regulation. The derived NOAEL value (100 mg/(kg bw x d)) from a two-generation reproduction toxicity study in rats was used as basis for the derivation of the long-term dermal DNEL value for workers. As assessment factors (AFs)[3] were applied a factor of 2 for time extrapolation, a factor of 1 for allometric scaling, a factor of 1 for remaining differences, a factor of 3 for intraspecies differences. The applied assessment factors deviate from the standard assessment factors recommended in the ECHA guidance document R.8 (ECHA, 2012). In the registration dossier, the observed non-specific systemic effects in the two-generation study and prenatal developmental toxicity study (see section 6.2.6) are judged as palatability issues. The various forms of the test substance (crystalline or molten) might explain differences in palatability, which were seen between the two-generation and repeated dose study. Because, in the opinion of the registrant, the main findings must be attributed to oral exposure, which is not relevant for workers, the registrant decided to apply a lower total assessment factor. The DNEL for the inhalation pathway is the general dust limit (ECHA Dissemination, 2020).

Table I.8: Overview of DNELs for systemic effects for workers by short- or long-term inhalation or dermal exposure

Route and duration of exposure	DNEL for workers	Study basis
Inhalation		
long-term	10.0 mg/m^3	General dust limit value TRGS 900, established by AGS (Ausschuss für Gefahrstoffe)
short-term	-	-
Dermal		
long-term	2,900 mg/(kg bw x d)	2-Generation study (OECD TG 416) in rats, oral Oral NOAEL = 100 mg/(kg bw x d) converted to dermal NOAEL = 17,333 mg/(kg bw x d) and AF = 6
short-term	-	-

Data taken from the registration dossier (ECHA Dissemination, 2020), AF = assessment factor

As already mentioned in section 6.1.2, the SCCP concluded on a maximum dermal absorption rate of 0.149% (SCCP, 2006; SCCP, 2008). In its 2008 safety assessment, the SCCP made a worst-case assumption for consumers that total exposure is composed of the value for total daily exposure to cosmetic products (17.79 g/d) and additional exposure from sunscreen products (18 g/d based on the value for sunscreen creams). Thus, in total 36g cosmetics per day (17.79 + 18 ≈ 36) containing 10% DHHB is assumed to be used by consumers. The systemic exposure dose (SED) and margin of safety (MoS, MoS = NOAEL/SED), based on a NOAEL from the prenatal developmental toxicity study in rats (see above section 6.2.6, NOAEL maternal= 200 mg/(kg bw x d)) were calculated. SED was calculated using total cosmetic exposure of 36 g, dermal absorption of 0.15%, and a body weight of 60 kg (SED = 3600 mg/d DHHB * 0.15%/60). The maximum SED was 0.09 mg/(kg bw x d) and the thereof calculated MoS (2,222) was regarded as safe (safe limit: MoS > 100) (SCCP, 2008). The safety evaluation only considered for dermal exposure and all other exposure routes are not taken into account.

In its MoS calculations for application of DHHB in sunscreens or other cosmetic products, the Danish EPA deviated from the SCCP in two factors, the dermal absorption expressed as percentage of test dose and the choice of the key

[3] AF (assessment factor) = Assessment factors are used to address the differences between the experimental data and the human situation, taking into account the uncertainties and variability in the extrapolation procedure and in the available data set

study and associated NOAEL. It used a value of 0.5% for dermal absorption (see section 6.1.1) and a NOAEL of 100 mg/(kg bw x d) identified in the two-generation study in rats (see above section 6.2.6). If sunscreen, which contains 10% DHHB, was applied daily with an amount of 36 g, the calculated MoS would be 330 (based on a SED of 0.3 mg/(kg bw x d)) and thus would be higher than the safety limit (MoS > 100), which means that no adverse health effects would be expected from this exposure. The application of other cosmetic products (aggregated amount of cosmetic products) with a daily applied amount of 17.4 g resulted in a MoS of 690, which is considered as safe (MoS > 100) (Danish EPA, 2015).

8 Selected biomarkers for DHHB

Within the scope of a cooperation project between the Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection (BMUV) and the German Chemical Industry Association (VCI) to support human biomonitoring, a biomonitoring method for DHHB in urine was developed (Stoeckelhuber et al., 2019). Stoeckelhuber et al. (2019) postulated the formation of three DHHB specific metabolites (see Figure 2):

- 2-(4-amino-2-hydroxybenzoyl)benzoic acid (amino-hydroxybenzoyl-benzoic acid, AHB, CAS No.: 67414-64-4),
- 2-(4-ethylamino-2-hydroxybenzoyl)benzoic acid (ethylamino-hydroxybenzoyl-benzoic acid, EHB, no CAS No. available), and
- 2-(4-diethylamino-2-hydroxybenzoyl)benzoic acid (diethylamino-hydroxybenzoyl-benzoic acid, DHB, CAS No.: 5809-23-4).

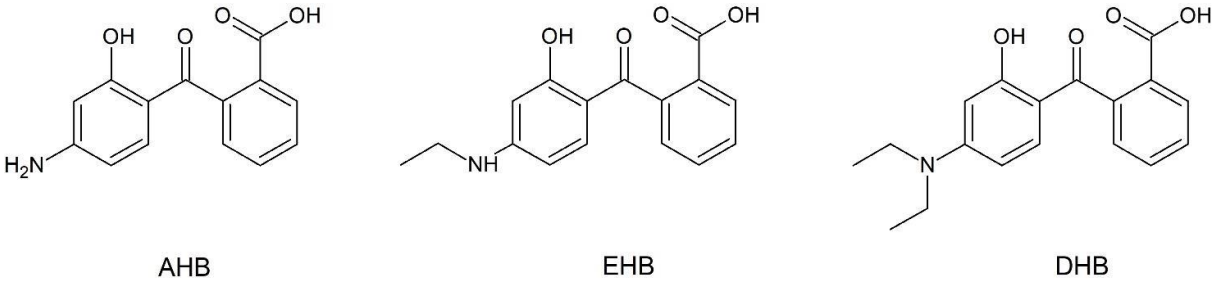


Figure I.2: Structural formulas of DHHB's metabolites AHB, EHB, and DHB (Stoeckelhuber et al., 2019)

DHB is formed by cleavage of the ester moiety of DHHB and has already been identified in rats (BASF unpublished, reported in Stoeckelhuber et al., 2019). The formation of the metabolites AHB and EHB by deethylation of the aniline function of DHHB has been postulated in correspondence to the metabolism of the structural analogue UV filter 2-ethylhexyl 4-(N,N-dimethylamino)benzoate (CAS No.: 21245-02-3). The detailed human metabolism of DHHB and urinary excretion of AHB, EHB and DHB has been published by Stoeckelhuber et al. 2020 (see chapter 6.1.1) providing essential urinary excretion fractions for these metabolites. A validated UPLC-MS/MS method (electrospray ionisation (ESI) positive mode and scheduled multiple reaction monitoring (MRM) mode) for the detection of all three postulated urinary metabolites was established according to guidelines from US Food and Drug Administration (FDA) (Stoeckelhuber et al. 2019). The lowest limit of quantification (LLoQ) was 0.1 µg/L for AHB and EHB and 0.05 µg/L for DHB, respectively. The validated method was used for the simultaneous determination of the three DHHB metabolites in spot urine samples of 58 healthy adult volunteers (31 males and 27 females, aged from 18 to 83) occupationally not exposed to DHHB. Highest urine concentrations were detected for EHB (average of 1.44 µg/L), followed by AHB (0.65 µg/L) and DHB (0.39 µg/L). All three metabolites were quantifiable in 26% of the samples. In 35% of the samples, at least one major metabolite could be quantified: samples with concentrations above the LoQ being 19 (33%) for AHB, 20 (36%) for EHB, and 14 (26%) for DHB. All three metabolites can be regarded as being specific metabolites for monitoring DHHB exposure and strongly correlate with each other (Spearman r = 0.81 - 0.92).

DHHB metabolites levels were not influenced by any factors, such as gender, age, daytime of sampling, season of sampling or age of spot urine (Stoeckelhuber et al., 2019; Stoeckelhuber et al., 2020). DHB, however, has the lowest urinary excretion fraction and the highest LOQ, making it less preferable for HBM purposes compared to AHB and EHB.

9 Derivation of HBM-GVs for DHHB with regard to the general population

The database for DHHB is not sufficient to derive an HBM-GV based on the relationship between human internal concentration of the exposure biomarker(s) and related health effects. Furthermore, no toxicity reference value for the general population suitable for the derivation of HBM-GVs was found. Therefore, the derivation of HBM guidance values is based on findings from toxicity studies on animals and additionally on human toxicokinetic studies. Critical effects that are considered relevant for the derivation of HBM-GVs are in particular effects on body weight (gain), observed in a two-generation reproduction study in rats (oral, feed) according to OECD TG 416. Since there is no data giving the indication for rodent-specific mechanisms, it can be assumed that these effects are also relevant for humans.

9.1 Choice of key study, POD and Assessment Factors

Due to the use of DHHB in sunscreen products and other cosmetics, skin exposure is considered to be the main route of exposure. It can be assumed that exposure peaks are the result of large-scale dermal application, but it is suspected that background exposure of the population is also oral (hand-mouth contacts, lipstick application) and possibly also inhalational (use of sprays). Dermal toxicity of DHHB was not investigated in toxicity studies in animals or humans.

A two-generation study in rats, which were exposed to Uvinul® A Plus via feed, was assessed by the registrant with a NOAEL for general parental toxicity at 100 mg/(kg bw x d), a LOEL for paternal toxicity (P1 males) at 100 mg/(kg bw x d) (based on "borderline reduction in food consumption and body weights in one paternal generation only"), a NOAEL for fertility and reproductive performance at 300 mg/(kg bw x d), and a NOAEL for developmental toxicity at 100 mg/(kg bw x d), for further details see section 6.2.6. Based on the available information in the registration dossier, the authors of this assessment agree with the registrant and do not consider the less than 10% reduction in mean body weight and the less than 10% reduction in body weight gain of P1 males at 100 mg/(kg body weight x d), concomitant with reduced food consumption, as adverse. In accordance with the registration dossier, the authors of this assessment also agree with the derived NOAELs for fertility and reproductive performance (300 mg/(kg bw x d)) and developmental toxicity (100 mg/(kg bw x d)). In a prenatal developmental toxicity study in rats the maternal NOAEL was 200 mg/(kg bw x d), but no developmental toxicity was observed at the highest tested dose (NOAEL: 1,000 mg/(kg bw x d)), for further details see section 6.2.6. The observed maternal effects were the same as seen in the two-generation study (decreased body weight (gain) and food consumption).

Overall, in *in vitro* and *in vivo* genotoxicity studies DHHB was found to be negative. There are no studies on carcinogenic effects available. The lowest NOAEL determined is 100 mg/(kg bw x d) due to general parental toxicity and developmental effects (growth and development of the offspring) in the two-generation study. This NOAEL is regarded as POD for the derivation of an external dose for the calculation of HBM-GVs. The key study is reliable without restrictions (1 (reliable without restrictions)), detailed investigations have been performed and it is a two-generation study, therefore it is considered relevant, despite the fact that in the developmental toxicity study (OECD TG 414) no developmental effects were observed.

As the pups were exposed during a sensitive stage of their development no time extrapolation factor is applied for the derivation of the external dose for the calculation of HBM-GVs. For interspecies differences a scaling factor of 4 (rat) and a factor of 2.5 for toxicodynamic differences and for intraspecies variability a factor of 10 was applied, thus in total the overall assessment factor was 100.

NOAEL: 100 mg/(kg bw x d) : 100 →

External dose (= TRV-like value) for the calculation of HBM-GV: 1 mg/(kg bw x d)

9.2 Calculation of HBM-GVs for the general population for DHHB

Stoeckelhuber et al. (2020) established an analytical method to determine three specific metabolites of DHHB in urine. However, equal treatment of all three metabolites for the derivation of HBM-values is not considered appropriate in this case, since such a procedure would require that all three metabolites have always to be quantified in urine in parallel. The metabolite DHB is not further considered, because it is the metabolite with the lowest urinary excretion fraction (5%) and the highest LOQ, and thus the metabolite with the lowest detection frequency.

For AHB (16%) and EHB (33%) one might consider using the sum of both for the derivation of a single HBM-GV. However, this would lead to a HBM-GV unduely dominated by EHB. Also, it would make it mandatory to always analyse both biomarkers. Therefore, a separate derivation of HBM-GVs for AHB and EHB is proposed. Exceeding either value is considered a sufficient indication of exceeding the HBM-GV for DHHB. In addition, the sum values are shown for information purposes.

By using the mass balance equation, assuming steady-state conditions, $HBM\text{-}GV_{GenPop\ (children\ and\ men)}$ of 3.5 mg/L for AHB and 7.9 mg/L for EHB, and $HBM\text{-}GV_{GenPop\ (women)}$ of 3 mg/L for AHB and 6.8 mg/L for EHB were calculated.

General formula: $$\frac{\text{TRV–like value} \cdot [\frac{\text{MW(Metabolite)} \cdot \text{Fue(Metabolite)}}{\text{MW(Substance)}}]}{\text{Daily urinary flow rate adjusted to the bw}}$$

TRV = toxicity reference value; MW = molecular weight; Fue = proportional urinary excretion factor (molar basis); bw = body weight

A TRV-like value of 1 mg/(kg bw x d), as well as urinary excretion factors (Fue) of 0.16 for AHB and 0.33 for EHB were used. The Fue values used are taken from the oral study by (Stoeckelhuber et al., 2020). No generally valid Fue can be derived from dermal studies because the application patterns (e.g. application area, frequency and time point of application) vary too much. In addition, as the TRV-like value is given in relation to body weight, the daily urine excreted is adjusted to body weight: 0.03 L/(kg bw x d) for children and men and 0.035 L/(kg bw x d) for women.

The values for adults were recently determined data-based by the German HBM Commission (German HBM Commission, 2025, submitted) and replace the originally by the German HBM Commission proposed and generally used default value for adults of 0.02 L/kg bw/d.

Table I.9 summarizes the input variables and resulting HBM-GVs.

The derived HBM-GVs represent the concentration of the metabolites in human urine up to which – for single substance evaluation – a risk for adverse health effects is not anticipated.

However, when assessing HBM results, it must be taken into account that exposure to mixtures of substances is usually present, so that the additional use of a MAF (mixture assessment factor/mixture allocation factor) or another suitable methodology should be considered for further assessments. At least structurally related substances or substances that display the same effect targets should be considered together for the next review and refinement of the HBM-GV.

Table I.9: Input variables for the derivation of HBM-GVs for DHHB

Urinary fraction	AHB: 16% EHB: 33%	Proportional amount (molar basis) excreted within 72 h
Molecular weight ratios	257.41/397.52 = 0.65 (AHB)	MW_{AHB}: 257.41
	285.43/397.52= 0.72 (EHB)	MW_{EHB}: 285.43
Body weight-related daily urine volume	0.03 L/(kg bw x d) for children and men 0.035 L/(kg bw x d) for women	
POD : TAF (total assessment factor)	100 mg/(kg bw x d): 100 = 1 mg/(kg bw x d)	TRV-like value, external dose and basis for HBM-GVs
Kinetic extrapolation and HBM-GV calculation for children and adults	AHB, children and men: 1 x 0.16 x 0.65 : 0.03 = 3.467 mg/L AHB, women: 1 x 0.16 x 0.65 : 0.035 = 2.971mg/L	Rounded values: AHB, HBM-GV children and men: 3.5 mg/L AHB, HBM-GV women: 3 mg/L
	EHB, children and men: 1 x 0.33 x 0.72 : 0.03 = 7.92 mg/L EHB, women: 1 x 0.33 x 0.72 : 0.035 = 6.789 mg/L	Rounded values: EHB, HBM-GV children and men: 7.9 mg/L EHB, HBM-GV women: 6.8 mg/L
		HBM-GV sum values for AHB and EHB, children and men: 11.4 mg/L, HBM-GV sum values for AHB and EHB, women 9.8 mg/L

9.3 Level of confidence attributed to the derived HBM-GVs

As mentioned in the HBM4EU strategy paper to derive HBM-GVs (Apel et al., 2020), it is suggested to attribute a level of confidence (low, medium or high) to each calculated HBM-GV. The level of confidence (LoC) should reflect the uncertainties identified during the calculation of the value and could constitute a good incentive to revise later on values with estimated 'lower' LoC.

Attributing low levels of confidence for the criteria described below may help to highlight the data gaps and to address them to the scientific research community. However, a low level of confidence does not necessarily mean a low level of protection, because the HBM-GV derivation is based on very conservative scenarios and default assumptions (Apel et al., 2020).

It should be noted that the LoC proposed in this document are not determined according to an established recognised methodology, but rather rely on expert judgment regarding the reliability of the data and the calculation method used to derive the HBM-GV values.

The overall LoC attributed to the $HBM\text{-}GV_{GenPop}$ are set to **'low'/'medium'** for DHHB

Please, refer to the n 2 note of the factsheet presented in section 10 for explanation on this LoC appreciation and to review the LoC for the single criteria that taken together define the overall LoC.

10 Factsheet HBM-GVs for DHHB for the general population

Table I.10: Data sheet for the derivation of HBM-GVs for DHHB

FACTSHEET for Diethylamino hydroxybenzoyl hexyl benzoate (DHHB; Uvinul A Plus) for the general population			
Parameter	**Note**	**Value / Descriptor**	**Comments**
$HBM\text{-}GV_{GenPop}$	1	AHB, HBM-GV children and men: 3.5 mg/L, AHB, HBM-GV women: 3 mg/L EHB, HBM-GV children and men: 7.9 mg/L, EHB, HBM-GV women: 6.8 mg/L Sum values for AHB and EHB, children and men: 11.4 mg/L, women 9.8 mg/L	
Level of confidence	2	Low/Medium	See note for explanation
Status	3	Agreed within PARC, 2025	
EC-No.	4	443-860-6	
CAS No	5	302776-68-7	
CLP classification	6	Aquatic Chronic, category 4 (H413)	
Molar mass	7	397.52 g/mol	
Identification exposure biomarkers	8	AHB EHB	
Molar mass of biomarker(s)	9	AHB: 257.41 g/mol EHB: 285.43 g/mol	
Key studies for TK data, authors, year	10	Stoeckelhuber et al., 2020, five healthy volunteers (three male, two female) received one single dose of 0.295 mg DHHB/kg bw in water/ethanol (100:1 v/v). The complete urine was collected before and for 72 hours, within these 72 hours, 54% of the administered dose was recovered in form of the three main metabolites: AHB, EHB and DHB, UPLC-MS/MS was used.	
Type of study	11	Human male and female volunteer study (n=5)	
Route of exposure	12	Oral administration	
Half-life of biomarker(s)	13	Urinary metabolite T1/2 (h) AHB: 4.5 EHB: 4.5	
Fraction urinary excreted (Fue(3d))	14	AHB: 0.16 (16%) EHB: 0.33 (33%)	
Derivation method	15	Based on animal POD	

FACTSHEET for Diethylamino hydroxybenzoyl hexyl benzoate (DHHB; Uvinul A Plus) for the general population			
Parameter	**Note**	**Value / Descriptor**	**Comments**
Key study, Author(s), Year	16		ECHA dissemination, 2020
Species	17	Male and female Wistar rats	
Route/type of study	18	Oral/2-generation toxicity study	
Exposure duration	19	Chronic	
Critical endpoint	20	General parental toxicity, developmental toxicity (mean absolute/relative spleen weights; decreased mean body weights of pups; impaired body weight gain of pups)	
POD Value	21	NOAEL: 100 mg/kg bw/d	
Assessment factors	22	(10 x 10)	Intra- and interspecies differences
TRV-like value	23	1.0 mg/kg bw/d	
Calculated HBM-GV_{GenPop}	24	AHB, HBM-GV children and men: 3.5 mg/L, AHB, HBM-GV women: 3 mg/L EHB, HBM-GV children and men: 7.9 mg/L, EHB, HBM-GV women: 6.8 mg/L Sum values for AHB and EHB, children and men: 11.4 mg/L, Sum values for AHB and EHB women: 9.8 mg/L	Mass balance approach

Explanation of note 2:

Level of confidence of the HBM-GV_{GenPop}: reflects the reliability of the proposed HBM-GV. The level of confidence takes into account the various uncertainties underlying the derivation of the HBM-GV (e.g. reliability of the key study used to derive the TRV/TRV-like value, uncertainties related to the extrapolations leading to the TRV/TRV-like value, to toxicokinetic data on the substance of interest, and to the calculation of the final HBM-GV). Attribution of a global level of confidence for the proposed DHHB HBM-GV_{GenPop} is suggested, considering:

- the nature and quality of the DHHB toxicological data: Information on toxicity to humans is limited to acute toxicity (ECHA dissemination, 2020). Therefore, the assessment of toxicity is based only on animal studies. A reproductive and additionally a developmental toxicity study on rats via the oral route of exposure have been conducted. As there is no reliable dermal toxicity study available, the oral 2-generation study was taken. The toxicokinetic data used for the HBM-GV calculation is based on one human volunteer study on only 3 males and 2 females, thus data on children or other sensitive population groups are lacking: **Low/medium confidence**
- the critical endpoint and mode of action: The evidence of the DHHB effects (reduced body weight (gain)) is based on animal studies via the oral route of exposure, this is adequate, since in addition to dermal exposure, human hand-mouth and lipstick exposure can also be assumed. There is no data giving the indication for a rodent-specific mechanism, thus it can be assumed that these effects are also relevant for humans. However, no evidence for these effects is available from epidemiological studies: **Low/medium confidence**
- the selected key study: 2-generation reproduction toxicity study in rats after oral administration of DHHB (ECHA dissemination, 2020) is reliable without restriction, but the original study is not available: **Medium confidence**

- the selected POD: NOAEL is given in the ECHA Dissemination (2020). Original study is not available: **Low confidence**
- the extrapolations leading to the TRV-like value: Default AFs for inter-species and intra-species variability: **Medium confidence**

Altogether the global level of confidence for the DHHB HBM-GVs in the general population is set to '**low/medium confidence**'.

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12 Annex on occupational exposure

Information taken from registration dossier

Manufacture

Environmental release category (ERC): ERC1: Manufacture of the substance

Contributing activity / technique for workers

Process category (PROC):

PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions

PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions

PROC 4: Chemical production where opportunity for exposure arises

PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities

PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities

PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing)

PROC 14: Tabletting, compression, extrusion, pelletisation, granulation

PROC 15: Use as laboratory reagent

PROC28: Manual maintenance (cleaning and repair) of machinery

Formulation

Formulation or packing

Environmental release category (ERC): ERC2: Formulation into mixture

Contributing activity / technique for workers

PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions

PROC 5: Mixing or blending in batch processes

PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities

PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities

PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing)

PROC 15: Use as laboratory reagent

PROC28: Manual maintenance (cleaning and repair) of machinery

Product category formulated:

PC 21: Laboratory chemicals

PC 28: Perfumes, fragrances

PC 39: Cosmetics, personal care products

Formulation into a mixture

Contributing activity / technique for workers

Process category (PROC):

PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions

PROC 5: Mixing or blending in batch processes

PROC 8a: Transfer of substance or mixture (charging and discharging) at non-dedicated facilities

PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities

PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing)

PROC 14: Tabletting, compression, extrusion, pelletisation, granulation

PROC 15: Use as laboratory reagent

PROC28: Manual maintenance (cleaning and repair) of machinery

Uses by professional workers

Use in cosmetics

Environmental release category (ERC):

ERC8b: Widespread use of reactive processing aid (no inclusion into or onto article, indoor)

ERC8d: Widespread use of non-reactive processing aid (no inclusion into or onto article, outdoor)

Process category (PROC):

PROC 10: Roller application or brushing

PROC 11: Non industrial spraying

PROC 19: Hand-mixing with intimate contact and only PPE available.

PROC 21: Low energy manipulation of substances bound in materials and/or articles

Part II

Substance monograph for (λ- and γ-) **cyhalothrin** and derivation of HBM guidance values (HBM-GVs) for the general population

Technical reference

Work package	**WP4 - Exposure and Monitoring**
Task	**Task 4.1 Human Biomonitoring**
Dissemination level 1	PU
Lead Beneficiary/ Responsible AE	UBA and VITO/ISCIII (T4.1)
Contributing Participants	Maria Tannous (ANSES) / maria.tannous@anses.fr Keyvin Darney (ANSES) / keyvin.darney@anses.fr Tamar Berman (MOH-IL)/ tamar.berman@moh.gov.il Lucija Perharic (NIJZ) / Lucija.Perharic@nijz.si
Responsible author(s)	Jens-Uwe Voss on behalf of UBA, Petra Apel (UBA), petra.apel@uba.de
Co-authors	/
Reviewers	Experts of NH Austria, NH Germany, NH Slovakia, NH Spain, NH Switzerland, NH United Kingdom, ECHA, EFSA Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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1 PU = Public
PP = Restricted to other programme participants (including the Commission Services)
RE = Restricted to a group specified by the consortium (including the Commission Services)
CO = Confidential, only for members of the consortium (including the Commission Services)

Document history

Version	Date receipt of opinion	Reviewer name/Institutions	Short description of changes
Version 1	Until 31.10.2023	Discussion within project, comments by P4.1.3.1.a partners	
Version 2	30.11.2023 - 17.01.2024	Austrian NH, German NH, Slovakian NH, Spanish NH, Swiss NH, UK NH, ECHA, EFSA	See Annex of AD 4.3
Version 3	16.12.2024 25.02.2025	Adoption by P4.1.3.1.a partners, comments considered from Tamar Berman (MOH-IL) Lucija Perharic (NIJZ)	See Annex of AD 4.3
Final Version	01.03.2025	Review of AD 4.3 by EAA	Update of 24h bodyweight-related urine volume for adults and recalculation of HBM-GVs for adults. Elaboration on ClF3CA as a major metabolite of cyhalothrin isomers and the pyrethroids bifenthrin and tefluthrin also sharing this metabolite. Unnecessary information deleted. Risk of exposure to substance mixtures shortly addressed.

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Abstract

Under PARC, WP4, project 4.1.3.1.a, human biomonitoring guidance values (HBM-GVs) are derived for priority substances. These toxicologically derived and within PARC jointly agreed HBM-GVs are to be used for direct comparison with the measured HBM data and are a valuable tool for the harmonised assessment of health risks. The human biomonitoring guidance value of a specific substance for the general population (HBM-GV_{GenPop}) represents the concentration of the substance or its relevant metabolite(s) in human biological material (mostly urine, less often blood or other tissues) up to which, according to current knowledge - when considering individual substances - there is no risk of adverse health effects. In the present case, HBM-GVs for the general population (HBM-GV_{GenPop}) were derived for (λ- and γ-) cyhalothrin whereby also the provisional values derived under HBM4EU were addressed. The HBM-GVs for the general population are for the specific urine metabolite ClF3CA (cis-3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropane-carboxylic acid) for children and men: 4.6 µg/L urine and for women: 3.9 µg/L urine[4]. While ClF3CA is a major metabolite of cyhalothrin isomers the pyrethroids bifenthrin and tefluthrin also share this metabolite. However, at the time of its approval as biocide, bifenthrin had only one registered product which was a wood preservative authorized in Austria, Denmark, France, Germany, Sweden and the Netherlands. Therefore, the exposure of the EU population to bifenthrin from its biocidal use can be considered very low and further decreases in the EU because bifenthrin is no longer approved within the EU as pesticide since 2019 and as biocide since January 2023. However, bifenthrin could be present in food products imported into the EU. Therefore, it is suggested that in individual cases in which a higher concentration of ClF3CA in urine (close to or exceeding the proposed HBM-GV) is measured, further investigations may be performed, including the determination of the bifenthrin-metabolite MPBA, to differentiate between exposure to cyhalothrin and bifenthrin. The other synthetic pyrethroid containing the ClF3CA moiety is tefluthrin. Principally, human exposure to tefluthrin can therefore be expected to lead to the excretion of ClF3CA in urine. However, tefluthrin is approved in the European Union only as insecticide in capsule suspension for seed coating performed in professional seed treatment facilities and in temporary emergency approvals for the laying of potatoes in the field for treatment against wireworms and click beetle larvae. No relevant consumer exposure is expected from these applications.

Key words

Human biomonitoring, cyhalothrin, λ-cyhalothrin, γ-cyhalothrin, ClF3CA, human biomonitoring guidance values, HBM-GV, risk assessment

[4] By using the outdated 24h bodyweight-related urine volume for men and women of 0.02 L/(kg bw x d) for the calculation of HBM-GV, the resulting HBM-$GV_{GenPop\ (adults)}$ would have been 6.9 µg/L.

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Authors and Acknowledgements

Authors

Jens-Uwe Voss on behalf of UBA,

Petra Apel (UBA), petra.apel@uba.de

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- NH Austria
- NH Germany
- NH Slovakia
- NH Spain
- NH Switzerland
- NH United Kingdom
- ECHA, EFSA

Comments were taken as far as possible into consideration for the finalisation of this report. An overview of the substantive comments and how they were dealt with can be found in the annex to this deliverable comprising various reports.

Background

Under PARC, WP4, project 4.1.3.1.a, human biomonitoring guidance values (HBM-GVs) are derived for priority substances. These toxicologically derived and within PARC jointly agreed HBM-GVs are to be used for direct comparison with the measured HBM data and are a valuable tool for the harmonised assessment of health risks. The HBM-GVs are derived as needed for the general population and/or occupationally exposed adults. There are three options for the HBM-GV derivation depending on the availability of data, their quality and their relevance in deriving HBM-GVs. The first and best option is the HBM-GV derivation from human data based on a relationship between internal concentrations and health effects. The second option to be considered is the HBM-GV derivation based on a defined external toxicological (toxicity) reference value (health-based guidance value - HBGV) and the third option is the derivation based on a critical effect observed in experimental (animal) studies (Apel et al., 2020).

The human biomonitoring guidance value of a specific substance for the general population (HBM-GV_{GenPop}) represents the concentration of the substance or its relevant metabolite(s) in human biological material (mostly urine, less often blood or other tissues) up to which, according to current knowledge - when considering individual substances - there is no risk of adverse health effects. This means that if the principle of back-calculation from the HBM-GV for the metabolite(s) to a previous exposure is applied, the possibly existing relevant HBGV in the population or in the geographical area concerned will most likely not be exceeded. In other words: If based on a health-based guidance value, an HBM-GV represents an internal exposure estimate at which the health-based guidance value selected as toxicological reference (in case of dietary exposure to pesticides, the Acceptable Daily Intake - ADI) is not exceeded and, thus, may be considered as safe from a health point of view.

An exceedance of the HBM-GV should be a reason to investigate the causes and to minimise possible sources of exposure.

In the present case, HBM-GVs for the general population (HBM-GV_{GenPop}) were derived for (λ- and γ-) cyhalothrin based on the specific urine metabolite CIF3CA, whereby also the provisional values derived under HBM4EU were addressed (Tarazona et al., 2022). The data base for cyhalothrin, including lambda- and gamma-cyhalothrin, is extensive and has been compiled in a number of reviews and evaluations (ATSDR, 2003; EC, 2012a; EC, 2013a; ECHA, 2011, 2023; EFSA, 2014a; EFSA, 2014b; EFSA, 2019; EFSA, 2020; EFSA, 2021; FAO and WHO, 2019; JECFA, 2000; JECFA, 2004; JMPR, 2007; U.S.EPA, 2007; WHO, 1990). A registration dossier according to REACH is also available for cyhalothrin, lambda-cyhalothrin (λ-cyhalothrin), and gamma-cyhalothrin (γ-cyhalothrin) (ECHA Dissemination, 2023a; 2023b; 2023c). For the preparation of this document, reports and evaluations prepared by recognised organisations have been accessed. Critical studies for the evaluation were, if available, checked against the original reference. However, most of the toxicokinetic and toxicity studies are studies performed by companies; these studies are not published but described in great detail in the assessment reports and evaluated by competent authorities within the scope of the approval processes.

HBM-GV_{Worker} were not derived by the authors of this report. The examination of whether an HBM-GV_{Worker} can be derived for (λ- and γ-) cyhalothrin and the corresponding work will be carried out in the extension period of the project P4.1.3.1.a by partners with expertise in the derivation of such values.

Results

Preparation of a substance dossier on lambda-/ gamma-cyhalothrin and derivation of HBM-GVs for the general population: ClF3CA, HBM-$GV_{GenPop\ –\ children\ and\ men}$: 4.6 µg/L urine; ClF3CA, HBM-$GV_{GenPop\ -\ women}$: 3.9 µg/L. urine

Abbreviations and acronyms

3-PBA	3-Phenoxybenzoic acid
ADI	Acceptable Daily Intake
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BUN	Blood Urea Nitrogen
bw	body weight
ClF3CA	Cis-3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropane-carboxylic acid, ((1R,3R)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylic acid; also called lambda-cyhalothric acid)
CFMP	Cis-3-(2-Chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethyl-cyclopropane-carboxylic acid
DCCA	3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanoic acid (= Cl2CAcid)
EFSA	European Food Safety Authority
F	Female
FAO	Food and Agriculture Organization
FOB	Functional Observational Battery
FPBA	4-Fluoro-3-phenoxybenzoic acid
GC-MS	Gas chromatography-mass spectrometry
GD	Gestation day
GerES	German Environmental Survey
HBGV	Health-based guidance value
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LD	Lactation day
LoD	Limit of detection
LoQ	Limit of quantification
M	Male
MoA	Mode of action
MPBA	2-Methyl-3-phenylbenzoic acid
MTBSTFA	N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamid
NHANES	National Health and Nutrition Examination Study
PCE	Polychromatic erythrocytes
POD	Point of Departure
RfD	Reference Dose

RMS	Rapporteur Member State: takes the lead in evaluating the application for authorization of plant protection products, coordinates with other member states within the same regulatory zone, known as "concerned member states" (cMS).
SCE	Sister chromatid exchange
SD	Standard deviation
UDS	Unscheduled DNA synthesis
UHPLC/Q-ToF MS	Ultra-high-performance liquid chromatography coupled to time-of-flight mass spectrometry
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

1 Substance properties

The structural formula of cyhalothrin is presented in Figure 1 and physicochemical properties of cyhalothrin are summarized in Table II.1. At room temperature, cyhalothrin has a very low vapour pressure and is nearly insoluble in water but very soluble in many organic solvents, e. g. methanol, acetone, hexane and toluene (PPDB, 2021a; PPDB, 2021b).

Table II.1: Physicochemical properties of cyhalothrin*

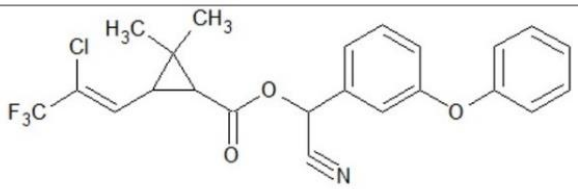
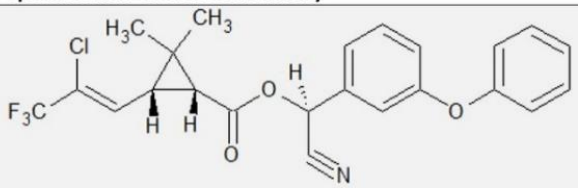
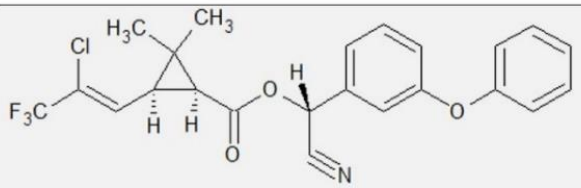
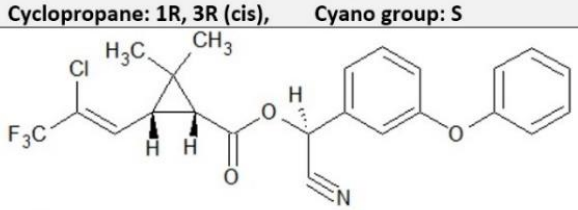
CAS No. EC-No.	Formula	Molar mass (g/mol)	Melting point (°C)	Boiling point (°C)	Vapour pressure at 20 °C (mPa)	log Pow	Solubility in water (µg/L)
Cyhalothrin, unspecified: 68085-85-8 - λ-cyhalothrin: 91465-08-6 415-130-7 γ-cyhalothrin: 76703-62-3 -	$C_{23}H_{19}ClF_3NO_3$	449.86	55.6	Decomposes before boiling	λ: 0.0002 γ: 0.00034	λ: 5.5 γ: 5.65	λ: 5 (at 20 °C) γ: 2 (at 20 °C)

* Data from ECHA Dissemination, 2023c; PPDB, 2021a; PPDB, 2021b

The cyhalothrin molecule has three chiral centers, two asymmetric centers in the acid moiety and one in the alcohol moiety, giving rise to four enantiomeric pairs and, principally, additionally Z-/E-isomers at the double bond of the vinyl group. However, technical grade cyhalothrin is composed only of the Z and cis forms, reducing the number of isomers.

Technical grade cyhalothrin is composed of four cis isomers in equimolar ratios. It is a yellow-brown viscous liquid (melting point: approximately 10 °C) containing at least 90 % active material (WHO, 1990). Lambda-cyhalothrin (λ-cyhalothrin) is the common name for the racemic mixture of (R)-α-cyano-3-phenoxybenzyl (1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate and (S) α-cyano-3-phenoxybenzyl (1R,3R)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxy-late (purity about 86 %, remainder are the two other isomers). Gamma-cyhalothrin (γ-cyhalothrin) is the designated name for the (S, 1R, 3R) isomer (also named (S)-α-cyano-3-phenoxybenzyl (Z)-(1R)-cis-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate).

The minimum purity is 810 g/kg for technical λ-cyhalothrin and 980 g/kg for γ-cyhalothrin (ECHA Dissemination, 2023c; EFSA, 2014a; EFSA, 2021; PPDB, 2021a; PPDB, 2021b). γ-cyhalothrin is the most insecticidally active isomer of cyhalothrin (and λ-cyhalothrin) and is also considered to be the most acutely toxic component of the cyhalothrin isomers (EC, 2012b; EFSA, 2014a).

Cyhalothrin	 Cl and cyclopropyl moiety in Z-position, no further specification of stereochemistry	
Lambda-cyhalothrin (1:1 mixture)	 Cyclopropane: 1R, 3R (cis), Cyano group: S	 Cyclopropane: 1S, 3S, Cyano group: R
Gamma-cyhalothrin	 Cyclopropane: 1R, 3R, cyano group: S	

IUPAC name of cyhalothrin (unstated stereochemistry): (RS)-α-cyano-3-phenoxybenzyl (1RS,3RS)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate (EFSA, 2014a)

Figure II.1: Structural formulas of cyhalothrin, λ-cyhalothrin and γ-cyhalothrin

2 Occurrence and use

The synthetic pyrethroid insecticide cyhalothrin is not authorized in Europe according to Regulation 1107/2009 (EU Pesticides Database - Active substances - Active substance details (europa.eu)), but the isomer λ-cyhalothrin was approved as an insecticide until 31/03/2024 (EU Pesticides Database - Active substances - Active substance details (europa.eu)) and the isomer γ-cyhalothrin is approved until 31/03/2025 (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/1211). λ-cyhalothrin is included in the list of candidates for substitution (EC, 2020) and is approved in the EU as active substance in biocidal products (product-type 18) until 31/03/2026 (according to COMMISSION IMPLEMENTING DECISION (EU) 2023/1087; ECHA, 2023).

3 Exposure and *human* biomonitoring

The major source of pesticide exposure of the general population is residues on food (https://www.hbm4eu.eu/wp-content/uploads/2022/07/Pesticides_Substance-report.pdf). This is also suggested by Morgan (2020) for pyrethroids including cyhalothrin and its isomers.

Inhalation exposure to (λ- or γ-) cyhalothrin vapours is not expected to a significant amount under environmental conditions due to its very low vapour pressure. However, in occupational settings, spray application of plant protection products could lead to (short-term) exposure against inhalable cyhalothrin aerosols.

4 Biomonitoring data

Urinary levels of the cyhalothrin metabolite cis-3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropanecarboxylic acid (ClF3CA) reflect recent exposure to cyhalothrin/λ-/γ-cyhalothrin but do not allow for further discrimination between isomer mixtures. The cyclopropane carboxylic acid metabolite ClF3CA is not strictly a specific metabolite of cyhalothrin but also of the synthetic pyrethroids bifenthrin and tefluthrin, so the detection of ClF3CA will reflect the combined exposure to the three compounds. However, tefluthrin is approved in the European Union only as insecticide in capsule suspension for seed coating performed in professional seed treatment facilities (EC, 2021a) and in temporary emergency approvals for the laying of potatoes in the field for treatment against wireworms and click beetle larvae (Proplanta, 2021). No relevant consumer exposure is expected from these applications. In a pilot study of human monitoring of eight metabolites of synthetic pyrethroids in urine of 38 individuals from the general population, ClF3CA could be quantified in 90% and the non-specific cyhalothrin metabolite 3-PBA (which is also produced from several other commonly used pyrethroids) in 100 % of the samples. The excretion of the sum of cyclopropane carboxylic acid metabolites (e.g. cis- and trans-DCCA, cis-DBCA, ClF3CA) was highly correlated with that of the 3-PBA excretion. On the other hand, the bifenthrin metabolite 2-methyl-3-phenylbenzoic acid (MPBA) could only be detected in 2 samples, indicating a rather scarce exposure to the parent pyrethroid bifenthrin (Schettgen et al., 2016). It is concluded that ClF3CA in urine reflects prior exposure to cyhalothrin, at least in Germany. Moreover, bifenthrin is currently not approved as insecticide in the European Union.

Results from biomonitoring studies measuring ClF3CA in urine are summarised in **Erreur ! Source du renvoi introuvable.**. In the pilot study of Schettgen et al. (2016), the concentration of ClF3CA in urine was above the LoQ of 0.01 µg/L in 90 % of the samples from the 38 adults included in the study. Detection frequency, and median (P50) of ClF3CA in urine were in a very similar range in a biomonitoring study performed in North Rhine-Westphalia, Germany on 247 children aged 2 – 6 years in the period 2017/2018. ClF3CA could be detected in most of the samples (94 %, LoQ 0.01 µg/L). The P50 and P95 were 0.06 and 0.29 µg/L, respectively, and the maximum value of 2.89 µg/L was about ten times higher than the P95 (LANUV, 2017).

Bevan et al. (2013) reported reference values for several biomarkers of chemical exposure within the UK population. Reference values reported represent 95th percentiles (P95) of the measured values. 92 were within accepted limits for urinary creatinine (between 0.3 and 3.0 g/L). The reported reference value (P95) of 3.2 µg ClF3CA/l urine is higher than the P95 values obtained in the German studies (LANUV, 2017; Schettgen et al., 2016) and falls within the maximum values reported in these two studies.

The results from the HBM4EU Aligned Studies show a widespread exposure to pyrethroids in the EU population. P95 of urinary CIF3CA concentrations are 0.679 (range: 0.514–1.02) µg/g crt across studies in children and 0.448 (0.386–0.542) µg/g crt across studies in adults. Further, 3-PBA was found above 90 % in most studies/data-collections (Andersen et al., 2022; Govarts et al., 2023).

Table II.2: Concentration and detection frequency of the cyhalothrin metabolite ClF3CA in urine

Study location, group (study period)	Number of samples	LoD/LoQ	Frequency of detection (%)	Concentration (µg/L)	Study
Germany, adults, 23 M, 15 F, 26-58 a (summer/autumn 2012)	38	LoQ: 0.01 µg/L	90	P50: 0.04 P95: 0.98 Max.: 4.86	Schettgen et al., 2016
Germany, children, 2-6 a (2017/2018)	247	LoQ: 0.01 µg/L	94	P50: 0.06 P95: 0.29 Max.: 2.89	LANUV, 2017
United Kingdom, adult general population (study period is not clearly stated, probably 2007 according to cross-reference)	92	LoD: 0.5 nmol/l (0.15 µg/L)	41	P50: n. r.* P95: 3.2 Max.: n. r.	Bevan et al., 2013
HBM4EU Aligned Studies, children, adults (2014 - 2021)	493 754	LoQ: 0.1-0.2 µg/L	45.6 27.1	In µg/g crt. P95: 0.679 P95: 0.448	Govarts et al., 2023

*: n. r.: not reported

In a recent Polish study (not listed in table II.2), 24-h urine samples were collected from 14 male and female volunteers (aged 7 – 68 a) and metabolites of eight active substances were measured once or twice per month over 12 consecutive months (Klimowska et al., 2020). ClF3CA could be detected in 39.3 % of all samples (n = 295); however, the median was below the LoD (0.1 µg/L) and the 90th percentile was 0.41 µg/L, the maximum reached 3.68 µg/L.

5 Toxicological profile

The toxicological database for cyhalothrin/λ-/γ-cyhalothrin, especially regarding animal studies, is very extensive; only the most relevant studies providing kinetic data necessary for the proposal of HBM values and toxicity data used for the derivation of tolerable doses will be reported here in more detail.

Most of the study reports have not been published but are reported in detail or condensed summaries in publications of competent authorities, e. g. assessment reports prepared according to European Commission Regulations, toxicological profiles of ATSDR, evaluations of the US EPA, and reviews from FAO/WHO. Generally, these studies were conducted in accordance with then-existing guidelines. The following summaries are largely those from European Agencies and FAO/WHO (EC, 2012a; EC, 2013a; ECHA, 2021; ECHA, 2024; EFSA, 2014a; EFSA, 2014b; EFSA, 2019; EFSA, 2020; EFSA, 2021; FAO and WHO, 2019; JECFA, 2000; JECFA, 2004; JMPR, 2007).

5.1 Toxicokinetics

A general overview of the metabolism of synthetic pyrethroids is presented in ATSDR (2003) and DFG (2008). Principally, the metabolites that have been identified in studies with mammalian animal species (especially rats) have also been identified in studies in humans. Thus, it is unlikely that there are significant qualitative differences between humans and other mammals in the major metabolic pathways for pyrethroids.

Synthetic pyrethroid compounds including cyhalothrin are degraded by similar metabolic processes in mammals. Biotransformation takes place through hydrolysis of the central ester bond, oxidative attacks at several sites, and conjugation reactions to produce a complex array of primary and secondary water-soluble metabolites that undergo urinary and biliary excretion. It is widely accepted that metabolism results in the formation of compounds that have little or no demonstrable toxicity, and it appears that cleavage of the ester bond results in substantial detoxification. Hydrolysis of the ester bond is followed by oxidation of the alcohol or cyanohydrin. Metabolites are excreted as alcohols, phenols, carboxylic acids, and their glycine, sulphate, glucuronide, or glucoside conjugates. Depending upon the type of pyrethroid compound, either oxidation or hydrolysis may predominate. The presence of the alpha-cyano group in type II pyrethroids such as cyhalothrin has been shown to decrease the rate of hydrolytic cleavage of the ester bond. In case of cyhalothrin, cleavage of the ester bond results in the formation of ClF3CA and a cyanohydrin. The latter rapidly releases cyanide, which is converted (and thus detoxified) mainly to thiocyanate ($S=C=N^-$), and 3-phenoxybenzyl aldehyde which is oxidized to 3-PBA. Metabolism studies with ^{14}C-labeled pyrethroids in general found little or no detectable amounts of $^{14}CO_2$, indicating that there appears to be little or no significant additional metabolic fragmentation of the acid and alcohol moieties (ATSDR, 2003; Hays et al., 2009; US EPA, 2019).

The results of animal studies, mainly with rats, but also with dogs, as well as studies involving humans (see below for details), and *in vitro* studies are summarised in the EC assessment reports for λ- and γ-cyhalothrin (EC, 2012a; EC, 2013a).

Overall, *"based on studies performed using lambda-cyhalothrin and cyhalothrin, it is predicted that gamma-cyhalothrin will be rapidly absorbed following oral administration (~50 – 80 % of the administered dose). Accumulation in fat is predicted; in a repeated dose study using cyhalothrin, an elimination half-life of approximately 23 days was reported. Extensive metabolism is predicted, with the initial metabolic step being inactivation of the compound by ester hydrolysis followed by oxidative metabolism and conjugation. Rapid urinary and faecal excretion is predicted. Although it is conceivable that the metabolism of gamma-cyhalothrin could be qualitatively or quantitatively different as a result of its structure, the comparable metabolism of cyhalothrin and lambda-cyhalothrin indicates that this is unlikely"* (EC, 2012a).

5.1.1 Human data

In an unpublished study summarised in the draft assessment report on λ-cyhalothrin (EC, 2013b) and by JMPR (2007), five male volunteers (a further was excluded from the study) received an oral dose of 5 mg λ-cyhalothrin in corn oil in a gelatine capsule followed by 150 ml of water. λ-cyhalothrin could be detected in blood plasma, indicating that the intact molecule can be absorbed and becomes systemically available, but the rapid occurrence of high concentrations of ClF3CA and 3-PBA in blood plasma indicated rapid hydrolysis of the molecule. λ-

cyhalothrin and ClF3CA could be detected in faeces, but, except in one volunteer, made up less than 1.5 % of the dose administered. Three (conjugated) metabolites were detected in urine: ClF3CA, 3-PBA and 4-hydroxy-3PBA at a concentration ratio of 2:1:1 (no data presented whether this represented molar or mass ratios). No parent compound was detected in urine. The three mentioned metabolites were typically detected in the first 4 hours after dosing and half-lives of elimination were 14 – 17 hours. The amount of λ-cyhalothrin orally absorbed was estimated to be 59 % on average (range: 50 – 64 %), based on the urinary excretion of ClF3CA. Excretion of λ-cyhalothrin metabolites after dermal application was also studied; however, no reliable determination could be achieved in this study (EC, 2013b).

The time course of cyhalothrin metabolites in blood and urine was studied in volunteers orally exposed to λ-cyhalothrin (Bouchard et al., 2018; Khemiri et al., 2017). All volunteers (5 M, 3 F, age 20 – 41 a) were healthy students who had not been exposed to pyrethroids during the three months period prior to the study, except perhaps via the diet. Three days prior to the study the participants abstained from fruits, vegetables or nuts; During the study they were given food prepared from organic ingredients to limit the ingestion of food potentially containing residues of pyrethroids. In a pre-study intended to verify whether the levels of metabolites were sufficiently high to establish a kinetic profile in plasma and urine one volunteer received a single dose of 0.0025 mg/kg bw/day (the U.S. RfD at the time then) in olive oil; since the levels of metabolites were almost non-quantifiable in plasma, the dose was therefore increased to 0.025 mg/kg bw/day for the main oral kinetic study with seven other volunteers. Blood samples were collected at 12 time points up to 72 h after dosing, and total urine voided was sampled 11 times for up to 84 h after dosing, respectively. Samples were spiked with 13C-labelled 3-PBA and 13C-D-labelled ClF3CA as internal standards and analysed for ClF3CA and 3-PBA by UHPLC/Q-ToF MS after enzymatic hydrolysis of glucuronide and sulphate conjugates. The limit of detection in plasma and urine was about 10 fmol injected in the UHPLC system[5].

The concentration of the metabolites ClF3CA and 3-PBA were below the limit of detection or very low[1] prior to dosing. After having received 0.025 mg/kg bw/day, ClF3CA and 3-PBA in blood plasma reached their peak values within 3.1 or 4.0 h, respectively. Both metabolites were rapidly cleared from blood plasma with apparent half-lives of elimination of 5.3 or 6.4 h, respectively (Bouchard et al., 2018; Khemiri et al., 2017).

At 0.025 mg/kg bw/day, time-to-peak levels in urine for ClF3CA and 3-PBA were 5.6 and 6.4 h, respectively, somewhat slower than the corresponding values for both metabolites in blood. The apparent half-life of elimination in urine was 4.2 h for ClF3CA and 5.9 h for 3-PBA, similar to the values observed in blood. The cumulative urinary excretion over the 84-h collection period, expressed as molar percentage of the orally applied cyhalothrin dose, was 21.3 ± 8.9 % in case of ClF3CA and 30.4 ± 9.4 % in case of 3-PBA (average ± SD, n=7). Most of the excretion occurred within the first 24 hours (ClF3CA: 20 %, 3-PBA: 22 %, as read from the graph presented in the publication). The percentage of dose recovered as ClF3CA in urine was considerably higher (59 % after 84 h) for the single person who had received the lower dose of 0.0025 mg/kg bw/day. However, the urinary concentrations of this metabolite were close to the limit of detection, hampering any further conclusions (Bouchard et al., 2018; Khemiri et al., 2017).

The same research group also conducted a toxicokinetic study with volunteers dermally exposed to λ-cyhalothrin (Bouchard et al., 2018; Khemiri et al., 2018). The six volunteers had participated in the oral kinetic study described above and followed a similar pre- and during-exposure nutrition protocol. They were dermally exposed to Matador©, a pesticide formulation containing λ-cyhalothrin in petroleum distillate (120 g/L). A dose equivalent of 0.25 mg λ-cyhalothrin/kg bw/day was applied to 40 cm^2 of the forearm for 6 h without coverage. Sampling and analysis of the metabolites ClF3CA and 3-PBA were performed as in the oral kinetic study described above.

The fraction of λ-cyhalothrin which had been dermally absorbed was rapidly cleared from the body. The calculated mean apparent plasma absorption half-lives for ClF3CA and 3-PBA were 3 and 7.3 h, respectively. The corresponding values for the mean apparent elimination from plasma were 11.2 and 7.6 h, indicating some on-site metabolism and storage of metabolites. Urinary excretion of the metabolites ClF3CA and 3-PBA was almost finished at 84 h after application. Overall, only 0.12 % and 0.08 % of the applied dose of λ-cyhalothrin was recovered in

[5] It is reported that 5 µl were injected. From these data and the molecular mass of ClF3CA of 242.6 g/mol a detection of about 0.5 µg/L can be calculated. The authors of the study also report that a concentration of 1.02 – 1.7 pmol/l (i.e., about 0.25 – 0.41 ug/L in case of ClF3CA) is "below the analytical limit of detection or very low" (Khemiri et al., 2017).

urine, indicating that the dermal absorption of λ-cyhalothrin is very low (Bouchard et al., 2018; Khemiri et al., 2018).

Very few other toxicokinetic data for cyhalothrin exposure in humans are available. Chester et al. (1992) estimated the uptake of cyhalothrin in 21 spray workers applying a wettable powder formulation containing 10 % λ-cyhalothrin for use in malaria vector control activities. No λ-cyhalothrin metabolites (ClF3CA and 3-PBA) could be detected in urine prior to starting the trial. The average daily excretion levels of cyhalothrin metabolites showed a slight upward trend over the days during the working week and sharply and rapidly declined thereafter; however, the data were insufficient to calculate elimination half-lives. The average amount of λ-cyhalothrin absorbed by the workers was 54 µg/d (< 1 µg/kg bw/day) for a 60-kg man. This represents less than 0.0001 % of the average daily amount of cyhalothrin handled (Chester et al., 1992).

5.2 Acute toxicity

5.2.1 Animal studies

The acute oral toxicity of cyhalothrin is moderate in rats and mice and low in guinea-pigs and rabbits (LD 50 values are as follows: rat, 144-243 mg/kg bw; mouse, 37-62 mg/kg bw; guinea-pig, > 5000 mg/kg bw; rabbit, > 1000 mg/kg bw). The acute oral toxicity of lambda-cyhalothrin is higher than that of cyhalothrin. In an oral toxicity study following OECD guideline 401 with **λ-cyhalothrin** applied in corn oil to fasting rats, LD50 values of 56 and 79 mg/kg bw were obtained for males and females, respectively. A lower LD50 value of 19.9 mg/kg bw was obtained in a similar study with male and female mice. The substance was less toxic after dermal application; The dermal toxicity (LD 50) for rats to cyhalothrin was 200-2000 mg/kg bw. LD50 values for λ-cyhalothrin in an OECD guideline 402 study with male and female rats were 632 and 696 mg/kg bw, respectively. However, λ-cyhalothrin was very toxic by inhalation (study in rats, 4 hours exposure "nose only" to aerosol with >66 % inhalable particles, LC50: 60 µg/L). The toxicity of λ-cyhalothrin was shown to be in the form of clinical symptoms typical of impaired motor function, regardless of the route of exposure. In addition, a slight treatment-related increase in lung weight was observed after inhalation, possibly related to the respiratory irritation observed in male rats after inhalation (WHO, 1990; EC, 2013a; 2013b).

Regarding **γ-cyhalothrin**, oral LD50 values of > 50 mg/kg bw in male rats and 55 mg/kg bw in female rats were obtained in an OECD guideline 401 study after oral application of the test substance as suspension in aqueous methylcellulose. The toxicity of γ-cyhalothrin was much lower when the test substance was applied as suspension in aqueous methylcellulose for 24 hours under semi-occlusive conditions to the skin of rats (OECD guideline 402 study); the dermal LD50 was > 1500 mg/kg bw. Deaths were noted at 1500 and 2000 mg/kg bw but not at 1000 mg/kg bw. γ-cyhalothrin was toxic by inhalation (rats, "nose only", inhalable aerosol, OECD 403 guideline study, 4-hour LC50 value: 40.2 µg/L) (2012a; EC, 2012b).

No signs of toxicity or deaths were noted in a study in which the inactive isomer of **λ-cyhalothrin** was given to Wistar rats at a dose level of 2000 mg/kg bw (as suspension in aqueous carboxymethylcellulose). This result indicates that γ-cyhalothrin is the principle toxic component of λ-cyhalothrin (EC, 2012b). This conclusion is supported by results of an acute neurotoxicity study (Moser et al., 2016) in which γ-Cyhalothrin was about 2-fold more active than λ-cyhalothrin.

It was concluded that *"lambda-cyhalothrin appears to be approximately three and two times more potent than cyhalothrin in rats and mice, respectively when comparing the acute oral toxicity of lambda-cyhalothrin reported in this dossier to the acute oral toxicity of cyhalothrin reported in literature." (EC, 2013a). In the assessment report for γ-cyhalothrin, it is concluded that "the comparative acute oral toxicity of the isomers is therefore broadly consistent with gamma-cyhalothrin being the toxicologically active component of cyhalothrin and lambda-cyhalothrin." (EC, 2012b). EFSA also concluded that γ-cyhalothrin shows "an overall higher acute toxicity than cyhalothrin or lambda-cyhalothrin"* (EFSA, 2014a).

5.2.2 Data on humans

Cyhalothrin (including λ- and γ-cyhalothrin) belongs to the class of type II pyrethroids. Generally, patients with ingestion of such pyrethroids can present with symptoms including, in mild cases, paraesthesia, nausea, vomiting, headache and fatigue which, at higher exposure, may progress to CNS depression, increased salivation,

fasciculations, fever, sweetening and blurred vision. Severe toxicity is characterised by seizures, coma, pulmonary oedema and respiratory failure (EC, 2012b; RAC, 2020). Chester (1992; cited in EC, 2012b) described symptoms like skin sensory effects, occasional coughing and sneezing in confined spaces among sprayers and handlers of 10% lambda-cyhalothrin.

In its 2018 report on pesticide residues in food, JMPR noted that in total 2,252 cases describing adverse effects relating to the use of λ-cyhalothrin had become known to the expert group. According to this report, occupational or accidental exposure generally led to temporary health effects of minor severity, and severe cases were related to intentional misuse (FAO and WHO, 2019). No information was provided in this summary on the exposure level.

Clinical symptoms arising from exposure at a research station and a formulation factory of a producer of λ-cyhalothrin (Syngenta) were reported to manifest as subjective facial sensation (skin-associated symptoms, including itching, tingling, burning, cold or numbness following dermal exposure) at all stages of λ-cyhalothrin handling. These symptoms may last for up to 24 hours after exposure, but recovery is apparently complete. The database reveals 223 cases of subjective facial sensation from cyhalothrin, 30 of these associated with eye irritation and 6 with headaches (ECHA, 2011). No information was provided on the exposure level.

5.3 Irritation and sensitisation

In a test following OECD guideline 404, γ-cyhalothrin was irritating to the skin of rabbits after a 4-hour semi-occlusive application. Irritation, persisting after one week, became visible as erythema, oedema, ulceration, flaking, scaling and scabbing at the application site (EC, 2012b).

γ-Cyhalothrin was also irritating to the eye of a rabbit (erythema and chemosis) in a test according to OECD guideline 405. The severity decreased over the next six hours after application of the test substance. At this timepoint the study was terminated, since the animal showed clear signa of acute neurotoxicity (repetitive grooming, head and nasal twitching, increased reactivity to stimuli and rapid respiration, chewing of paws) (EC, 2012b).

Eye irritation (chemosis, redness, and discharge) was also observed in a study following OECD guideline 405 after instillation of λ-cyhalothrin into rabbit eyes. All symptoms disappeared within 10 days or earlier. At most slight initial pain was observed immediately after application into the eye, but signs of paraesthesia were noted during the first 24 hours of the study in all animals. No other toxic effects were observed (EC, 2013a; 2013b).

A skin sensitisation maximisation study following OECD guideline 406 was performed with γ-cyhalothrin in guinea pigs. A sensitisation rate over more than 70 % was observed in this study. Accordingly, there was clear evidence of skin sensitisation for the test material under the conditions of this study (EC, 2012b). Regarding λ-cyhalothrin, the (negative) results of two maximisation studies with guinea pigs were regarded as not conclusive, mainly because the challenge or the induction dose was too low (EC, 2013a; EC, 2013b). Technical-grade cyhalothrin has been reported to cause skin sensitization in a Buehler test and a maximization test in guinea-pigs (JMPR, 2007).

U.S. EPA (2015) concluded in 2015, based on the analyses at that time, that there is no clear and consistent pattern of effects reported to indicate conclusively whether there is an association between pyrethrins/pyrethroid exposure and asthma and allergies. The Agency did not require additional warnings or label statements specific to asthmatic or allergic individuals on pyrethroids and pyrethrins end-use products, nor did the Agency require additional data from pyrethroid registrants.

5.4 Repeated dose toxicity

5.4.1 Animal studies

Studies have been conducted with rats, mice, and dogs . For an overview see Table II.3.

Short- and medium-term toxicity

Studies were conducted with rats and dogs.

Studies with rats

In a subacute oral toxicity study with Wistar **rats** (10 M + 10 F/group) (following OECD guideline 407), the animals were fed diets with 0, 2.5, 10, 50, 125, or 250 ppm **γ-cyhalothrin** for **28 days**. No mortality was observed, but the high-dose group was terminated after five days due to excessive neurotoxicity (incoordination; postural abnormalities including crouching, tip-toe gait and knuckling; splayed limbs, rigid limbs, arched back, limp/flaccid muscles, piloerection, perineal/perioral/abdominal soiling) and weight loss. Neurotoxicity was also observed at 125 ppm but not at 50 ppm. At 125 ppm, signs of toxicity included increased activity and reactivity, crouched posture, splayed hindlimbs, tip-toe gait, knuckling, noisy and laboured breathing, incoordination, salivation and lacrimation. No such signs were noted at 50 ppm. This concentration represented the **NOAEL** (equivalent to **4.2 and 4.5 mg/kg bw/day** in males and females, respectively) based on the clinical signs, a reduced weight gain and food consumption and alterations of clinical-chemical parameters (BUN, AST, ALT increased, protein, albumin, creatinine and calcium lowered) at 125 ppm (EC, 2012b).

Reversibility of liver changes were studied in male Wistar **rats**. The animals were fed diets containing 250 ppm **cyhalothrin** (about 12.5 mg/kg bw/day) for **28 days**, followed by a recovery period of 7, 12, or 28 days (8 rats per group). The body weight gain was reduced during treatment and until sacrifice. The relative liver weight was unchanged. Histopathological examination revealed a proliferation of the smooth endoplasmic reticulum in only five exposed rats at termination of treatment, but not in rats of the recovery groups. Hepatic aminopyrine-N-demethylase activity was increased at exposure termination but not seven days later (JMPR, 2007).

In a subchronic study (following OECD guideline 408), 10 M + 10 F Wistar **rats**/group received diets containing 0, 2.5, 10, 50, or 100 ppm (equivalent to 0, 0.2/0.2, 0.7/0.8, 3.4/4.2, 6.6/8.0 mg/kg bw/day in M/F) **γ-cyhalothrin** (purity 99.1 %) for **13 weeks**; an additional control and high-dose group were examined after a 4-week recovery period. Clinical signs of toxicity (decreased extensor-thrust response, incoordination, palpebral closure, piloerection, decreased reactivity and responsiveness to touch), effects on body weight, slight changes in haematology and clinical chemistry and increased liver and kidney weights were observed at 100 ppm (**NOAEL: 50 ppm**, equivalent to **3.4 and 4.2 mg/kg bw/day** in males and females, respectively (EC, 2012b).

In a similar subchronic study (following OECD guideline 408 except that there was no recovery group) with λ-cyhalothrin (purity 89.2), Wistar **rats** (20 M + 20 F/group) were fed diets containing 0, 10, 50, or 250 ppm **λ-cyhalothrin** (equivalent to 0, 0.5, 2.6 and 13.6 mg/kg bw/day for males and 0.6, 3.2 and 15 mg/kg bw/day for females) for **13 weeks.** A **NOAEL** for male and female rats of **10 ppm (0.5 and 0.6 mg/kg bw/day** for males and females, respectively) was obtained in this study, based on elevated hepatic aminopyrine-N-demethylase activity at 50 ppm and decreased body weight gain and decreased food consumption at the top dose (EC, 2012b).

In a second, similarly conducted subchronic study with λ-cyhalothrin (following OECD guideline 408 except that there was no recovery group), Wistar **rats** (20 M + 20 F/group) received diets with 0, 10, 50, or 250 ppm **λ-cyhalothrin** (purity 96.5 %) (equivalent to about 0, 0.7, 3.3 and 16.7 mg/kg bw/day for males and females). Different NOAELs were reported for males and females: The **$NOAEL_{males}$** was **10 ppm (0.7 mg/kg bw/day)**, based on an increased relative liver weight and an elevated aminopyrine-N-demethylase activity in the liver at 50 ppm, and reduced body weight gain and reduced food consumption at 250 ppm. The **$NOAEL_{females}$** was **50 ppm (3.3 mg/kg bw/day)**, based on similar changes as observed in males but only at 250 ppm (EC, 2012b).

Table II.3: Overview of relevant repeated dose oral toxicity studies with (λ-/γ-) cyhalothrin

Species, strain, sex, no./group	Substance, exposure, concentration in food in ppm or dose in mg/kg bw/d	Exposure duration	LOAEL (mg/kg bw/day or ppm)	NOAEL (mg/kg bw/day or ppm)	Effect at LOAEL	OECD guideline Ref.
Rat, Wistar, 10 M, 10 F	**γ-Cyhalothrin**, diet: 0, 2.5, 10, 50, 125, or 250 ppm	28 d	125 ppm	4.2 (50 ppm)	increased activity and reactivity, crouched posture, splayed hindlimbs, tip-toe gait, knuckling, noisy and laboured breathing, incoordination, salivation and lacrimation, reduced weight gain and food consumption, and alterations of clinical-chemical parameters (BUN, AST, ALT increased, protein, albumin, creatinine and calcium lowered)	OECD guideline 407 (EC, 2012b)
Rat, Wistar, 8 M	**Cyhalothrin,** diet: 0, 250 ppm	≤ 28 d ≤ 28 d recovery	-	-	body weight gain reduced during treatment and until sacrifice; liver: proliferation of endoplasmatic reticulum and increase of amino-pyrine-N-demethylase activity at end of exposure, but not at end of recovery (reversible), other effects not studied	(JMPR, 2007)
Rat, Wistar, 10 M, 10 F	**γ-Cyhalothrin,** diet: 0, 2.5, 10, 50, 100 ppm	13 weeks	M: 6.6, F: 8 mg/ kg bw/day (100 ppm)	M: 3.4, F: 4.2 mg/ kg bw/day (50 ppm)	clinical signs of neurotoxicity, reduced weight gain, slight changes in haematology and clinical chemistry, increased liver and kidney weight	OECD guideline 408 (EC, 2012b)
Rat, Wistar, 20 M, 20 F	**λ-Cyhalothrin,** diet: 0, 10, 50, 250 ppm	13 weeks	M: 2.6, F: 3.2 mg/ kg bw/day (50 ppm)	M: 0.5, F: 0.6 mg/ kg bw/day (10 ppm)	elevated hepatic aminopyrine-N-de-methylase activity	OECD guideline 408 except for lack of a recovery group (EC, 2012b)

Species, strain, sex, no./group	Substance, exposure, concentration in food in ppm or dose in mg/kg bw/d	Exposure duration	LOAEL (mg/kg bw/day or ppm)	NOAEL (mg/kg bw/day or ppm)	Effect at LOAEL	OECD guideline Ref.
Rat, Wistar, 20 M, 20 F	**λ-Cyhalothrin,** diet: 0, 10, 50, 250 ppm	13 weeks	M: 3.3 mg/kg bw/day (50 ppm) F: 16.7 mg/kg bw/day (250 ppm)	M: 0.7 mg/kg bw/day (10 ppm) F: 3.3 mg/kg bw/day (50 ppm)	increased liver weight, elevated hepatic aminopyrine-N-de-methylase activity	OECD guideline 408 except for lack of a recovery group (EC, 2012b)
Rat, Alpk/AP, 62 M, 62 F	**Cyhalothrin,** diet: 0, 10, 50, 250 ppm	2 a	250 ppm	M: 1.7, F: 1.9 mg/ kg bw/day (50 ppm)	decreased weight gain, increased relative liver weight, clinical parameters	OECD guideline 453, except for the low number of survivors in all groups at study termi-nation (EC, 2012b; EC, 2013a)
Mouse, CD-1, 52 M, 52 F	**Cyhalothrin,** diet: 0, 20, 100, 500 ppm	2 a	100 ppm	M: 1.8, F: 2.0 mg/ kg bw/day (20 ppm)	hunched posture, piloerection	OECD guideline 453 (EC, 2012b; EC, 2013a)
Dog, Beagle, 1 M, 1F	**λ-Cyhalothrin**, capsules with corn oil: 0, 0.75, 1.5, 3.0, 4.0 mg/kg bw/day	6 weeks	1.5 mg/kg bw/day	0.75 mg/ kg bw/day	neurotoxicity (tremor or unsteady gait, decreased activity, increased salivation)	(EC, 2013a)
Dog, Beagle, 12 M, 12 F	**Cyhalothrin,** capsules with corn oil: 0, 1, 2.5, 10 mg/kg bw/day	26 weeks	10 mg/kg bw/day	2.5 mg/kg bw/day (systemic)	neurotoxicity (excessive salivation, vomiting and incoordination)	OECD guideline 409, increased exposure duration (EC, 2012b)

Species, strain, sex, no./group	Substance, exposure, concentration in food in ppm or dose in mg/kg bw/d	Exposure duration	LOAEL (mg/kg bw/day or ppm)	NOAEL (mg/kg bw/day or ppm)	Effect at LOAEL	OECD guideline Ref.
Dog, Beagle, 6 M, 6 F	**λ-Cyhalothrin,** capsule with corn oils: 0, 0.1, 0.5, 3.5 mg/kg bw/day	1 a	3.5 mg/kg bw/day	0.5 mg/kg bw/day (systemic)	neurotoxicity (ataxia, muscle tremors, convulsions)	OECD guideline 409, increased exposure duration (EC, 2013a)

Studies with dogs

No studies with γ-cyhalothrin were reported in the assessment reports.

A **6-week** oral toxicity pilot study was performed with **λ-cyhalothrin** (purity 87.7 %) in Beagle **dogs** (1 M + 1 F/group). The animals received the test substance in capsules with corn oil (2 h after feeding) at doses of 0, 0.75, 1.5, 3.0 or 4.0 mg/kg bw/day for six weeks. Both animals at the highest dose were killed on day 15 due to excessive toxicity (inappetence, body weight loss and clinical signs including decreased activity). Neurotoxicity was also observed at 1.5 and 3.0 mg/kg bw/day (tremor or unsteady gait, decreased activity, increased salivation at one or both doses). Also, regurgitation occurred in all dogs at doses ≥ 1.5 mg/kg bw/day. Fluid faeces were occasionally seen in all treated groups. The **NOAEL** was determined at **0.75 mg/kg bw/day** based on neurotoxicity at 1.5 mg/kg bw/day (EC, 2013a).

Beagle **dogs** (12 M + 12 F/group) received (one hour before feeding) capsule preparations of **cyhalothrin** in corn oil at doses of 0, 1, 2.5, or 10 mg/kg bw/day for **26 weeks** (following OECD guideline 409, but with increased exposure duration). No deaths occurred. A dose-related increase in the passage of liquid faeces were noted in all groups receiving the test compound. Excessive salivation, vomiting and incoordination and a transient increase in water consumption occurred occasionally in dogs receiving 10 mg/kg bw/day. There were no treatment-related effects on any other parameters (haematology, clinical chemistry, urine parameter, organ weights and histopathology including the nervous system). The assessment report noted that the increased incidence of liquid faeces could be related to the method of administration (capsule preparation) and not to the test substance (**NOAEL: 2.5 mg/kg bw/day** based on clinical signs of neurotoxicity at 10 mg/kg bw/day) (EC, 2012b). JMPR concluded that the LOAEL for local gastrointestinal effects was 1.0 mg/kg bw/day, based on the increased incidence of liquid faeces at the lowest dose (no NOAEL for this effect) (JMPR, 2007).

A **one-year** oral toxicity study was performed with lambda-cyhalothrin (purity not specified) in Beagle **dogs** (following OECD guideline 409, but with increased exposure duration). 6 M + 6F/group received **λ-cyhalothrin** dissolved in corn oil (capsule preparation, given one hour before feeding) at doses of 0, 0.1, 0.5, or 3.5 mg/kg bw/day for **52 weeks.** At the highest dose, one male dog was killed after 46 weeks due to adverse clinical effects consistent with pyrethroid toxicity. The other dogs at this dose also showed clinical signs of neurotoxicity (ataxia, muscle tremors and convulsions). At the highest dose, food intake was also slightly lower, plasma triglyceride levels were slightly decreased and plasma cholesterol increased. Fluid faeces were more commonly observed at doses ≥ 0.5 mg/kg bw/day. There was no evidence of any treatment-related effects on bodyweight gain, gross or microscopic changes or histopathological changes in the nervous system. Based on clinical effects indicating neurotoxicity, the **NOAEL** was determined at **0.5 mg/kg bw/day** (EC, 2013a). JMPR also concluded that the NOAEL for systemic toxicity was 0.5 mg/kg bw/day, based on neurotoxic effects at 3.5 mg/kg bw/day. However, a NOAEL of 0.1 mg/kg bw/day was reported for local gastrointestinal effects, based on the increased incidence of liquid faeces at 0.5 mg/kg bw/day (JMPR, 2007).

Long-term Toxicity

Studies were performed with oral administration of cyhalothrin via diet to rats and mice. No long-term toxicity studies using λ- or γ-cyhalothrin are available. Structural considerations, comparable metabolism, and comparable short-term effects justify a read across between cyhalothrin and λ-/γ-cyhalothrin. However, differences in potencies should be considered if NOAELs derived for cyhalothrin are applied to lambda or γ-cyhalothrin in the risk assessment (EC, 2012a; EC, 2013a).

In the study with Wistar-derived **rats** (Alpk/AP), 62 M + 62 F/group were fed a diet containing 0, 10, 50, or 250 ppm **cyhalothrin** for **two years**. Interim sacrifices with additional 10 M + 10 F were conducted after 52 weeks. The treatment had no effect on mortality and clinical signs. Compared with the control group, the body weight was lower in the high dose group (up to 11%), and a decreased body weight gain was accompanied by a small decrease in food consumption during the first 12 weeks of the study. At the highest dose, small changes in blood biochemistry were also observed (lowered blood glucose, cholesterol and triglyceride concentrations). The relative liver weight was increased at the highest concentrations, but no histopathological changes occurred in the liver or other organs. No adverse effects were observed at **50 ppm** cyhalothrin in the diet (**NOAEL**) (JMPR, 2007). The study was conducted in accordance with OECD guideline 453 (except for the low number of survivors in all groups at study termination). Pituitary adenomas were observed in all groups, including controls, and thus this observation was not considered to be related to treatment. These adenomas and a marked rhinitis with associated oro-nasal fistulation which was attributed to the presence of fibrous particles in the diet increased the mortality rate (survival was less than 50 % at termination of the study) and consequently reduced the reliability of the study. According to the assessment reports for λ- and γ-cyhalothrin, the concentration of 50 ppm cyhalothrin in food corresponds to a dose of approximately 1.7 and 1.9 mg/kg bw/day in male and female rats, respectively (EC, 2012b). (According to JMPR, 50 ppm corresponds to a dose of 2.3 mg/kg bw/day (JMPR, 2007)**Erreur ! Signet non défini.**.

In the corresponding study (following OECD guideline 453) with **mice**, 52 M + 52 F CD-1 mice/group were treated with 0, 20, 100, or 500 ppm **cyhalothrin** in their diet for **two years**. Interim sacrifices with additional 12 M + 12 F were conducted after 52 weeks. The treatment had no effect on mortality. At 500 ppm, males and females showed piloerection and hunched posture; males additionally showed emaciation, pallor, hyperactivity, increased fighting activity, reduced food efficiency and reduced bodyweight gain and increased water intake. Males also showed hunched posture and piloerection at 100 ppm. No dose-related histological or weight changes of organs related to treatment were observed (for carcinogenicity, see below). No adverse effects were observed at **20 ppm** cyhalothrin in the diet (**NOAEL**) (JMPR, 2007). According to the assessment reports for λ- and γ-cyhalothrin, the concentration of 20 ppm cyhalothrin in food corresponds to a dose of approximately 1.8 and 2.0 mg/kg bw/day in male and female rats, respectively (EC, 2012b; EC, 2013a). (According to JMPR, 20 ppm corresponds to a dose of 1.8 mg/kg bw/day (JMPR, 2007).

5.4.2 Data on humans

The U.S. EPA prepared a Tier II Epidemiology Report focused on carcinogenic and non-carcinogenic health effects in humans for the chemical class of pyrethroids, as well as selected individual pyrethroid chemicals including cyhalothrin, λ- and γ-cyhalothrin (US EPA, 2019).

A systematic literature review was conducted and 62 out of almost 600 epidemiological studies investigating the association between exposure to pyrethroids and carcinogenic and non-carcinogenic human health effects were analysed in detail. It is stated that in general, many of the published studies are of low quality and relied on cross-sectional designs in which exposure measures and outcome measures are determined at the same time. Such designs are generally considered inferior to cohort or case-control studies in which a temporal sequence is actually established. Pyrethroid exposure usually was estimated from biomonitoring data on the excretion of pyrethroid metabolites, especially cis- and trans DCCA, 3-PBA and, in some studies, also 4FPBA. If exposure to cyhalothrin was monitored, the non-specific metabolite 3-PBA was measured but not the more-specific cyhalothrin metabolite ClCF3CA. The review covered a broad range of health outcomes including several cancers, neurodevelop-mental/neurobehavioral/neurocognitive effects, birth effects, male reproductive effects, and a variety of more general other effects. The conclusions regarding cancer, reproductive outcomes and neurotoxicity are presented in the corresponding chapters.

Other outcomes were only examined in a small number of studies (one to three at most). Overall, there is "no evidence at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure for the following health outcomes: neurologic effects, suicide, hearing loss, and diabetes" and "insufficient evidence at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure for the following health outcomes: coronary heart disease in adults, depression, amyotrophic lateral sclerosis, end-stage renal disease, glucose abnormalities, myopia, sleep apnea, and the respiratory effects wheeze, asthma, and chronic bronchitis" (US EPA, 2019). In order to better classify this statement, it must be mentioned once again that the analysed studies only examined associations.

A study not included in the EPA evaluation was conducted on adults who participated in the US NHANES conducted from 1999 to 2002 and provided urine samples for pyrethroid metabolite measurements (mostly 3-PBA). No data for the cyhalothrin metabolite ClF3CA were available for this study. After adjustment for various parameters (age, sex, race/ethnicity, socioeconomic status, dietary and lifestyle factors, body mass index, and urinary creatinine levels), the hazard ratios for all-cause mortality and cardiovascular disease mortality among participants with the highest tertile compared with those with the lowest tertile of urinary 3-PBA levels were 1.56 (95%CI, 1.08-2.26) and 3.00 (95%CI, 1.02-8.80), respectively. The authors concluded that further studies are needed to replicate the findings (Bao et al., 2020).

5.5 Genotoxicity

In vitro studies with λ-cyhalothrin with and without S9 metabolic activation included mutagenicity assays in bacteria (various strains of *Salmonella typhimurium*) and mammalian cells (L5178Y (TK +/-) mouse lymphoma cell assay), a cytogenicity assay for chromosomal aberrations in human primary lymphocytes and a test for UDS in primary cultures of rat hepatocytes. λ-cyhalothrin did not induce mutations and did not increase the frequency of chromosomal aberration or UDS in these tests. λ-cyhalothrin also was not clastogenic *in vivo* in a micronucleus test in mice at a single oral dose of 22 and 35 mg/kg bw, respectively. All tests followed the corresponding OECD guidelines (471, 473, 476, 482) except the *in vivo test* in which the number of polychromatic erythrocytes (PCE) examined was only 500 instead of 1000 PCE. Based on the overall data, it was concluded that *"lambda-cyhalothrin does not possess any mutagenic or clastogenic properties*" (EC, 2013a; EC, 2013b).

In the evaluation of λ-cyhalothrin, JMPR noted that several published studies, largely from the same laboratory, reported positive results of comet assays *in vitro* and of chromosomal aberrations *in vitro* and *in vivo*. According to JPMR, the materials tested in these studies were either commercial formulations of unknown composition or were inadequately described. Considering the uniform lack of any genotoxicity in studies with adequately characterized λ-cyhalothrin, JMPR concluded that *"lambda-cyhalothrin is unlikely to be genotoxic"* (JMPR, 2007).

γ-cyhalothrin was not genotoxic in *in vitro* studies following the corresponding OECD guidelines with and without S9 metabolic activation in mutagenicity assays in bacteria (various strains of *Salmonella typhimurium*) and mammalian cells (L5178Y (TK +/-) mouse lymphoma cell assay) and in a cytogenicity assay for chromosomal aberrations with primary rat lymphocytes. γ-cyhalothrin also was not clastogenic *in vivo* in a micronucleus test in mice at a single oral dose of 22 and 35 mg/kg bw, respectively (again, the number of polychromatic erythrocytes (PCE) examined was only 500 instead of 1000 PCE). γ-cyhalothrin was thus not considered to be genotoxic (EC, 2012b).

5.6 Carcinogenicity

5.6.1 Animal studies

Chronic toxicity/carcinogenicity studies have been conducted with cyhalothrin in rats and mice (see under: "Repeated dose toxicity, animal studies, long-term toxicity"). The assessment report for λ-cyhalothrin concluded that *"no long-term toxicity studies using lambda-cyhalothrin are available. Based on structural considerations, comparable metabolism, and comparable short-term effects read across between the two substances is considered justified."* (EC, 2013a). A similar statement was made in the assessment report for γ-cyhalothrin (EC, 2012b).

In the **rat** study, pituitary adenomas were observed in all groups, including controls, and thus this observation was not considered to be related to treatment. No evidence of carcinogenicity of cyhalothrin was apparent in this study (EC, 2012b; EC, 2013a),

In the chronic toxicity/carcinogenicity study with cyhalothrin in **mice**, an increased incidence of mammary adenocarcinomas was noted in females at the mid and the high dose of cyhalothrin (100 and 500 ppm). The mammary adenocarcinoma developed late in the study with no pre-neoplastic changes and the effects were similar to the historical control. Whereas the assessment for λ-cyhalothrin concluded that the observed adenocarcinoma could be related to treatment (EC, 2013b), the assessment report for γ-cyhalothrin did not support this conclusion. This report stated that *"findings were only seen in the later stages of the study and were not associated with pre-neoplastic changes. The tumour incidences in the two affected groups (100 and 500 ppm) are not dose-related (13.5 % and 11.5 % respectively) and are comparable to the reported historical control range (2 – 12 %). The concurrent control incidence is low (1.9 %) and lies outside the historical control range. … It is therefore concluded, on the basis of structural considerations, comparable metabolism, the negative chronic toxicity studies with cyhalothrin and the negative genotoxicity studies with gamma-cyhalothrin, that there is no evidence of carcinogenic potential for gamma-cyhalothrin"* (EC, 2012b).

Based on the overall weight of evidence, EFSA also concluded for λ- and γ-cyhalothrin that "no genotoxic or carcinogenic potential was observed" (EFSA, 2014a; EFSA, 2014b).

5.6.2 Data on humans

A total of four studies evaluated in the U.S. EPA Tier II Epidemiology Report investigated the relationship between exposure to pyrethroids and carcinogenic outcomes including childhood brain tumors, childhood leukemia, and breast cancer. The U.S. EPA concluded that for *"childhood brain tumors and childhood leukemia, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposures."* Furthermore, *"there is **no evidence** at this time to conclude that there is a clear associative or causal relationship between pyrethroid exposure and breast cancer."* (US EPA, 2019). In order to better classify this statement, it must be mentioned once again that the analysed studies only examined associations.

In a study not included in the EPA evaluation, no increased hazard ratio for cancer mortality was observed (0.91 (95%CI, 0.31-2.72)) after adjustment for various parameters (Bao et al., 2020).

5.7 Toxicity to reproduction

5.7.1 Animal studies

Multi-generation study

The study (largely following OECD guideline 416, except for the reporting of historical control incidences) was conducted with cyhalothrin. Wistar **rats** (15 M, 30 F) received cyhalothrin (purity 89.2 %) in their diets at dietary concentrations of 0, 10, 30 or 100 ppm for three successive generations. Two litters (named "a" and "b) were reared in each generation, the second litter was used to produce the next generation. Males from each generation were killed after completion of mating; female parents were sacrificed after weaning the "b"-litter. All parent animals were fully examined post mortem. All offspring from "a"-litters and the pups from "b"-litters that were not selected as next generation parents were sacrificed on day 36 after birth and subjected to gross autopsy or a full post mortem examination ("b"-litters) (EC, 2012b; EC, 2013b; JMPR, 2007).

Body-weight gain was slightly reduced (up to 9 %) in parental animals at the highest dose, particularly in males. A slight reduction in body-weight gain (7 %) was also observed at 30 ppm cyhalothrin, but only in F1-females. Reductions in pup weight gain during lactation (up to −17 %) were observed at 100 ppm in all but the F2a generation. A slight reduction of the litter size at the highest dose was also noted (considered not toxicologically significant by JMPR (2007)). Other parameters (food consumption, fertility rate, duration of pre-coital period, duration of gestation) were not affected by treatment. No effects on organ histology or neurological effects were seen in any generation (JMPR, 2007).

In the assessment report, the NOEL/NOAEL for both, parental animals and pups, was reported to be 30 ppm (about 1.5 - 1.9 mg/kg bw/day), based on reduced parental body weight gain, and a small decrease in litter weight and litter weight gain noted at 100 ppm (EC, 2013b).

However, although not explained in detail by EFSA (2014a, b), this dose seems to have been regarded as LOEL/LOAEL in the evaluation and the derivation of the ADI for λ- and γ-cyhalothrin by EFSA and the **NOAEL for offspring is reported as 0.5 mg cyhalothrin/kg bw/day, based on reduced body weight gain** (2014a, b). In accordance with this, the EC reported that *"the lowest relevant NOAEL (0.5 mg/kg bw/day)* was *seen in the multigenerational study in rats"* (EC, 2020).

The RMS also noted that a "detailed testicular histopathological examination and a quantitative evaluation of primordial follicles and sperm parameters (sperm morphology and motility) as described in OECD TG 416 (adopted 22nd January 2001) were not performed" (EC, 2013b). However, based on a systematic review thereby requested by the EFSA to assess the evidence available as regards potential sperm effects linked to exposure to lambda-cyhalothrin, EFSA concluded that "none of the reliable studies provided any evidence on potential sperm effects linked to the exposure to lambda-cyhalothrin" (EC, 2020).

Developmental toxicity

Teratogenicity studies with oral administration of cyhalothrin and γ-cyhalothrin were conducted in rats and rabbits.

In a dose-finding study, pregnant CD **rats** (8/group) received 0, 1, 2.5, 5, 10, or 15 mg/kg bw/day **γ-cyhalothrin** in corn oil by gavage on GD 6 – 20. All dams administered > 5 mg/kg bw/day showed excessive signs of toxicity (soiling, incoordination, slow breathing, tremor, ptosis) and were killed on days 7 or 8. Some signs of neurotoxicity were apparent at 2.5 mg/kg bw/day (uncoordinated gait, soiling). Weight gain and food consumption were significantly lowered at 2.5 mg/kg bw/day and non-significantly (except at GD 9 – 12) at 1 mg/kg bw/day. No effect of treatment was seen on litter or foetal parameters (EC, 2012b).

In the main study (following OECD guideline 414), pregnant CD **rats** (24/group) received 0, 0.1, 0.5 or 2.0 mg **γ-cyhalothrin**/kg bw/day in corn oil by gavage on GD 6 – 20. Clinical signs of toxicity similar to that in the pilot study and reduced weight gain were noted at 2.0 mg/kg bw/day. The proportions of resorptions were higher in all treated groups in an apparently treatment-related fashion, but were within the historical control range at the same laboratory. Also, litter size and weight were slightly, but not significantly lower than controls at the highest dose. No treatment-related effect was seen on the incidence of malformations or teratogenic effects. A higher incidence of a variation (extra lumbar ribs) was noted at the highest dose but was not considered as clearly related to treatment. The assessment report reported a **$NOAEL_{maternal}$** of 0.5 mg/kg bw/day and a **$NOAEL_{developmental}$** of **2.0 mg/kg bw/day** (EC, 2012b).

A study (following OECD guideline 414) was also conducted with oral administration of 0, 5, 10, or 15 mg **cyhalothrin**/kg bw/day in corn oil to pregnant CD **rats** (24/group) on GD 6 – 15. Based on neurotoxic effects (loss of limb coordination) and a reduced weight gain, a **$NOAEL_{maternal}$** of **10 mg/kg bw/day** was obtained in this study. No treatment-related embryo-/fetotoxicity or teratogenicity was observed up to the highest dose (EC, 2012b; JMPR, 2007).

In a study (following OECD guideline 414 with minor deviations regarding housing conditions of the animals) with pregnant New Zealand White **rabbits** (18 – 22/group), 0, 3, 10, or 30 mg **cyhalothrin**/kg bw/day in corn oil was administered by gavage on GD 6 – 18. Initial weight loss, reduced weight gain and food intake were noted at the highest dose. Other treatment related effects on the dams were not observed. No treatment-related embryo-/fetotoxicity or teratogenicity was observed up to the highest dose. The **$NOAEL_{maternal}$** was **10 mg/kg bw/day** (EC, 2012b; JMPR, 2007).

5.7.2 Data on humans

A review of pyrethroid epidemiology concluded that pyrethroids as a group are unlikely to adversely affect human fertility or reproductive capability (Burns and Pastoor, 2018).

The U.S. EPA concluded in 2019 that for neurodevelopmental effects, birth defects, male reproductive effects with respect to semen quality, male reproductive hormone levels, and sperm damage/genetic abnormalities "*overall*

there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship" between any of the endpoints listed above and pyrethroid exposure (US EPA, 2019). In order to better classify this statement, it must be mentioned once again that the analysed studies only examined associations.

A more recent study by Andersen et al. (2022), on the other hand, concludes that pyrethroids are probably developmental neurotoxicants and adverse impacts of pyrethroid exposure on neurodevelopment are likely at exposure levels occurring in the general population. The authors of this study evaluated the epidemiological evidence for neurodevelopmental effects related to prenatal and childhood pyrethroid exposure by means of a systematic review and assessed biological plausibility by evaluating mechanistic evidence. Twenty-five epidemiological studies met the inclusion criteria: 15 presented findings following prenatal exposure only, 8 on childhood exposure, and two took both exposure windows into consideration. The overall body of evidence was rated as "moderate quality" with "sufficient evidence" for an association between prenatal pyrethroid exposure and adverse neurodevelopment. For childhood exposure, the overall rating was "low quality" with "limited evidence" because of cross-sectional study design.

5.8 Neurotoxicity studies

5.8.1 Animal studies

An overview of relevant studies is presented in Table II.4**Erreur ! Signet non défini.**.

In an **acute** neurotoxicity study (following OECD guideline 424) with λ-cyhalothrin given in corn oil by gavage to **Wistar** rats (10 M + 10 F/group; 0, 2.5, 10, 35 mg/kg bw), the following effects were observed at the highest dose of 35 mg/kg bw at approximately 7 hours and/or 24 hours post-dosing: decreased activity, ataxia, increased breathing rate, diarrhoea, reduced stability and/or tiptoe gait, salivation, piloerection, upward curvature of spine and/or urinary incontinence, tremor, changes in the FOB, and reduced motor activity. Slight changes of breathing rate and of single FOB parameters in individual animals at 10 mg/kg bw were considered as not adverse. A **NOAEL** of **10 mg/kg bw** was reported based on neurotoxicity (EC, 2013a).

The relative potency for acute effects of pyrethroids (including λ-cyhalothrin) on motor function was studied in **rats**. Adult male Long Evans rats (8 – 18/dose) received 0.015 to 15 mg λ-cyhalothrin/kg bw in corn oil by gavage (9 different doses). Motor function was measured 2.5 h after dosing during a period of 1 h in a maze test. Relative potencies were calculated based on computed ED30, i.e., the dose required to induce a 30 % decrease in total motor activity. For λ-cyhalothrin an ED30 of 1.32 ± 0.13 (SE) mg/kg bw and a **threshold dose** of **0.52 ± 0.13 (SE) mg/kg bw** was determined (Wolansky et al., 2006).

A similar study design was used in a further study with Male Long Evans **rats** to compare the potency differences for **λ-** and **γ- cyhalothrin** on motor activity (Moser et al., 2016). For the dose-response studies, the following doses (vehicle and six doses) were used: specifically, λ-cyhalothrin 0.188, 0.375, 0.75, 1.5, 3, 6 mg/kg bw (n = 9/dose, except that one rat in the 1.5 mg/kg group had wounds from fighting and was excluded) and γ-cyhalothrin 0.0938, 0.188, 0.375, 0.75, 1.5, 3 mg/kg bw (n = 10/dose). Both compounds were administered in corn oil by gavage. Motor activity was recorded in an activity chamber after dosing. Initial time-course studies showed that the time of peak effect was 1.5 h for both isomers. Rats were sacrificed immediately after the end of the 1-h session and cyhalothrin concentrations in brain and blood were determined.

Both isomers produced dose-related decreases in activity counts. The differences were statistically significant from control at doses ≥ 0.75 mg/kg bw for both, λ- and γ-cyhalothrin. Based on the administered dose, γ-cyhalothrin was about 2fold more active than λ-cyhalothrin: The calculated doses to decrease motor activity by 50 % **(ED50)** was **1.29 mg/kg bw for γ-cyhalothrin** (brain level: 13.37 ng/g; plasma level: 486.1 ng/ml) and **2.65 mg/kg bw for λ-cyhalothrin** (brain level: 21.11 ng/g; plasma level: 615.5 ng/ml). Administered doses correlated well with the tissue concentration of the corresponding cyhalothrin in brain and plasma which were analysed using LC/MS/MS methods. The differences based on internal concentrations were less, with γ-cyhalothrin being 1.3- to 1.6-fold more potent than λ-cyhalothrin (Moser et al., 2016).

Table II.4: Overview of relevant neurotoxicity studies with (λ- and γ-) cyhalothrin

Species, strain, sex, no./ group	Substance, exposure, concentration in food or dose	Exposure duration	LOAEL (mg/kg bw or ppm)	NOAEL (mg/kg/d or ppm)	Effect	Ref.
Rat, Wistar, 10 M, 10 F	**λ-Cyhalothrin,** gavage: 0, 2.5, 10, 35 mg/kg bw	1 d	35 mg/kg bw	10 mg/kg bw	Decreased activity, ataxia, increased breathing rate, diarrhoea, reduced stability and/or tiptoe gait, salivation, piloerection, upward curvature of spine and/or urinary incontinence, tremors	EC, 2013a
Rat, Long-Evans, male 8 – 18/dose	**λ-Cyhalothrin,** gavage, corn oil: 9 doses (0.015 – 15 mg/kg bw)	2.5 h	ED30: 1.32 mg/kg bw	Threshold: 0.52 mg/kg bw	Dose-dependent decrease in motor activity by 50%	Wolansky et al., 2006
Rat, Long-Evans, male 10/ dose	**γ-Cyhalothrin,** gavage, corn oil: 0, 0.0938, 0.188, 0.375, 0.75, 1.5, 3 mg/kg bw	1.5 h	ED50: 1.29 mg/kg bw		Dose-dependent decrease in motor activity by 50% (with γ-cyhalothrin being 1.3- to 1.6-fold more potent than λ-cyhalothrin based on internal concentrations)	(Moser et al., 2016)
Rat, Long-Evans, male 8 or 9/dose	**λ-Cyhalothrin,** gavage, corn oil: 0, 0.188, 0.375, 0.75, 1.5, 3, 6 mg/kg bw	1.5 h	ED50: 2.65 mg/kg bw		Dose-dependent decrease in motor activity by 50%	(Moser et al., 2016)
Rat, Wistar, 30 pregnant F	**λ-Cyhalothrin,** diet: 0, 25, 60, 150 ppm from GD7 – LD22 (gestation: 0, 2.1, 4.9, 11.4, lactation: 0, 4.6, 10.7, 26.3 mg/kg bw/day)	GD7 – LD22	Maternal: 11.4 mg/kg bw/day Develop-mental neuro-toxicity: - Offspring: 26.3 mg/kg bw/day	Maternal: 4.9 mg/kg bw/day Develop-mental neuro-toxicity: ≥ 11.4 mg/kg bw/day Offspring: 10.7 mg/kg bw/day	Maternal: reduced weight gain Developmental neurotoxicity: - Offspring: reduced pup survival and body weight during lactation	(EC, 2013a; EC, 2013b)

A developmental neurotoxicity study with λ-cyhalothrin was conducted in rats (EC, 2013a; EC, 2013b). Pregnant Wistar-derived rats (30/group) received diets with 0, 25, 60, or 150 ppm λ-cyhalothrin from GD7 to LD22. The concentrations in the diet corresponded to 0, 2.1, 4.9, or 11.4 mg/kg bw/day during gestation and 0, 4.6, 10.7, 26.3 mg/kg bw/day thereafter. No treatment-related clinical signs or effects in the FOB were noted. Maternal bodyweight, weight gain and food intake were reduced at ≥ 60 ppm. In F1 animals, survival to day five and body

weight were slightly reduced at the highest dose. Motor activity or auditory startle were not affected. Swimming performance on day 21 was reduced in females at the highest dose. The **$NOAEL_{maternal}$** was **4.9 mg/kg bw/day**, the **$NOAEL_{developmental\ neurotoxicity}$** was **≥ 11.4 mg/kg bw/day**. The **$NOAEL_{offspring}$** was reported to be 60 ppm **(10.7 mg/kg bw/day)** based on the reduced pup survival and reduced bodyweights during lactation. The assessment report noted deficiencies in the study due to deviations from the OECD guideline 426 (especially, no neuropathological analyses at intermediate and low dose groups although evidence of neuropathological alterations noted at the high dose group).The reliability of the study was therefore regarded as limited (EC, 2013a). There was an increased incidence of demyelination in the proximal sciatic and distal tibial nerves of males at 150 ppm on day 63. This is a common spontaneous finding in this age and strain of rat, and the increased incidences were within the ranges for historical controls and therefore were considered to be incidental.

A delayed neurotoxicity study with cyhalothrin, λ- or γ-cyhalothrin is not available. The assessment report for λ-cyhalothrin noted that *"since lambda-cyhalothrin is a synthetic pyrethroid and does not belong to a class of chemistry (organophosphates) known to be associated with delayed neurotoxicity following acute exposure such a study is not required"* (EC, 2013a).

5.8.2 Data on humans

The U.S. EPA concluded that "overall, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and neurologic symptoms." (US EPA, 2019). In order to better classify this statement, it must be mentioned once again that the analysed studies only examined associations: Two studies investigated the association between pyrethroid exposures and neurologic symptoms in adults. A third study evaluated the association between urinary pyrethroid metabolites and neurologic symptoms among female farmers. All three studies are cross-sectional studies.

A more recent study by Andersen et al. (2022), on the other hand, concludes that pyrethroids are probably developmental neurotoxicants and adverse impacts of pyrethroid exposure on neurodevelopment are likely at exposure levels occurring in the general population (see chapter "developmental toxicity").

5.9 Endocrine effects

EFSA concluded that *"lambda-cyhalothrin is not classified or proposed to be classified as carcinogenic category 2 or toxic for reproduction category 2, in accordance with the provisions of Regulation (EC) No 1272/2008, and therefore the conditions of the interim provisions of Annex II, Point 3.6.5 of Regulation (EC) No 1107/2009 concerning human health for the consideration of endocrine disrupting properties are not met.* ***However, an endocrine-mediated mode of action could not be ruled out*** *regarding the brain morphological changes observed in the developmental neurotoxicity study (and possible sperm effects, which have to be clarified in the first place) and the potential for endocrine disrupting effects could not be finalised"* (EFSA, 2014b).

Regarding sperm effects, EFSA concluded in a more recent evaluation that *"none of the reliable studies provided any evidence on potential sperm effects linked to the exposure to lambda-cyhalothrin"* (EC, 2020).

A study in Brazil on 122 male and female adults residing in small agricultural properties reported an association between recent use of λ-cyhalothrin and a reduced total T4 and increased male luteinizing hormone (LH) level in blood serum. Cyhalothrin exposure (or its concentration in blood or of one of its metabolites in urine) was not measured in this study but was concluded from questionnaire (Santos et al., 2019).

Four pyrethroids (deltamethrin, permethrin, sumithrin (phenothrin), and bifenthrin) were evaluated for the list of endocrine disruptors by the Danish Centre on Endocrine Disrupters. Sumithrin was reported to lack endocrine effects, while deltamethrin and bifenthrin were regarded as "suspected" endocrine disruptors. Cyhalothrin was not evaluated in this report (Hass et al., 2017).

There is ED concern also for the environment (https://pubmed.ncbi.nlm.nih.gov/32806430/, https://www.sciencedirect.com/science/article/abs/pii/S0269749120321345) and from in vitro studies which demonstrate disturbance of the endocrine system (eg: https://pubmed.ncbi.nlm.nih.gov/29506456/)

6 Classification proposals and toxicological reference values

λ-Cyhalothrin is listed in Annex VI of Regulation (EC) No. 1272/2008 (CLP Regulation) as "Acute Tox 3 (H301)", "Acute Tox. 4 (H312)" and "Acute Tox. 2 (H330)" (harmonized classification) (ECHA C&L Inventory, 2021). λ-cyhalothrin is not classified or proposed to be classified as carcinogenic category 2 or toxic for reproduction category 2 (EC, 2020).

λ-Cyhalothrin is approved as biocide until 31.03.2026 (ECHA, 2011, ECHA, 2023)

JMPR concluded that, "in view of the lack of genotoxicity of lambda-cyhalothrin and the absence of carcinogenicity shown by cyhalothrin in mice and rats, lambda-cyhalothrin is unlikely to pose a carcinogenic risk to humans" (JMPR, 2007). No evaluation of IARC is available regarding the carcinogenicity of cyhalothrin (IARC, 2020).

Table II.5: Toxicity (toxicological) reference values (TRVs, increasingly named HBGVs) for λ- and/or γ-cyhalothrin for the protection of the general population at oral exposure

Species, end-point/ target organ, study type	POD [mg/kg bw/day]	AF Time	AF Inter-species	AF Intra-species	AF other	AF Total	TRV [mg/kg bw/day]	Reference
Rat, reduced body weight gain in offspring, multi-generation study, largely following OECD guideline 416	NOAEL: 0.5		10	10	2	200	ADI, λ-cyhalothrin: 0.0025	(EC, 2020; EFSA, 2014b)
Rat, reduced body weight gain in offspring, multi-generation study	NOAEL: 0.5		10	10	4	400	ADI, γ-cyhalothrin: 0.0012	(EC, 2021b; EFSA, 2014a)
Rat, acute neurotoxicity; dog, one-year study	NOAEL: 0.5					25	ADI, λ-cyhalothrin: 0.02	(JMPR, 2007; FAO and WHO, 2019)
Dog, liquid faeces, neurotoxic signs[6] one-year oral toxicity study	0.1		10	10	3	300	ADI, λ-cyhalothrin: 0.0003	(Health Canada, 2021)
Rat, acute neurotoxicity	$BMDL_{1SD}$ 0.28		10	10	3	300	aRfD, λ-cyhalothrin: 0.0009	(Health Canada, 2021)

[6] EC (2013a) determined the NOAEL neurotoxicity at 0.5 mg/kg bw/day. JMPR also concluded that the NOAEL for systemic toxicity was 0.5 mg/kg bw/day, based on neurotoxic effects at 3.5 mg/kg bw/day. However, a NOAEL of 0.1 mg/kg bw/day was reported for local gastrointestinal effects, based on the increased incidence of liquid faeces at 0.5 mg/kg bw/day (JMPR, 2007).

Species, end-point/ target organ, study type	POD [mg/kg bw/day]	AF Time	AF Inter-species	AF Intra-species	AF other	AF Total	TRV [mg/kg bw/day]	Reference
Rat, acute neurotoxicity	$BMDL_{1SD}$ 0.28		10	10		100	aRfD, λ-cyhalothrin: 0.0028	(U.S. EPA, 2020; U.S. EPA, 2017)
Rat, acute neurotoxicity	$BMDL_{1SD}$ 0.18		10	10		100	aRfD, γ-cyhalothrin: 0.0018	(U.S. EPA, 2020; U.S. EPA, 2017)
Dog, neurotoxicity	0.5					100	ADI, cyhalothrin: 0.0050	(CVMP, 2001)

The ADI of 0.0025 mg/kg bw/day for **λ-cyhalothrin** in the EC pesticide risk assessment is based on the NOAEL of 0.5 mg/kg bw/day for a decrease in body weight gain, obtained in the multigeneration study with cyhalothrin in rats. A total uncertainty factor of 200 was used, consisting of a standard factor of 100 and an additional factor of 2 to convert from cyhalothrin to λ-cyhalothrin, taking into account the higher toxicity of the latter (EFSA, 2014b).

The ADI of 0.0012 mg/kg bw/day for **γ-cyhalothrin** is based on the same NOAEL as for λ-cyhalothrin, but using an uncertainty factor of 400, i.e. a standard factor of 100 and an additional factor of 4 to convert from cyhalothrin to γ-cyhalothrin (EFSA, 2014a). A detailed explanation and a justification for the selection of the NOAEL is not presented in both EFSA publications.

For **λ-cyhalothrin**, the JMPR established a common ADI and acute RfD of 0.02 mg/kg bw/day. This value was based on the NOAEL of 0.5 mg/kg bw/day for λ-cyhalothrin obtained for acute neurotoxicity in a study with rats and also in a 1-year toxicity study with dogs in which neurotoxicity occurred a few hours after dosing during the first week of treatment. The NOAEL was considered to be protective of other non-neurotoxic effects from studies of repeated doses. It was further considered appropriate to apply a chemical-specific adjustment factor of 25 for this end-point, leading to the derived values of 0.02 mg/kg bw/day (JMPR, 2007). The ADI of 0.02 mg/kg bw/day was later confirmed after the evaluation of additional toxicological studies, concluding that the new studies did not have any impact on the derivation of the value (FAO and WHO, 2019).

The ADI of 0.0003 mg/kg bw/day for **λ-cyhalothrin** derived by Health Canada (2021) is based on a NOAEL of 0.1 mg λ-cyhalothrin/kg bw/day obtained in the one year oral toxicity study with dogs (liquid faeces, neurotoxic signs at the LOAEL of 0.5 mg/kg bw/day[3]). The composite assessment factor of 300 consists of a standard factor of 100 and an additional database uncertainty factor of 3. Health Canada (2021) also derived an acute RfD for acute dietary exposure of 0.0009 mg/kg bw/day, based on reduced motor activity in rats at the time-of-peak observed in two acute oral non-guideline neurotoxicity studies with λ-cyhalothrin (Moser et al., 2016; Wolansky et al., 2006). A BMDL of 0.28 mg/kg bw/day (no details available regarding the calculation) obtained from the data of the Moser study served as the POD for the derivation of the ARfD, also using a composite assessment factor of 300.

The derivation of the acute RfDs for **λ-cyhalothrin** and **γ-cyhalothrin** of the U.S. EPA are also based on the acute neurotoxicity studies in rats (Moser et al., 2016; Wolansky et al., 2006) in which the **decrease in total motor activity** (ED30) was assessed. EPA also performed a benchmark calculation to derive a $BMDL_{1SD}$ of 0.28 mg/kg bw/day for λ- and of 0.18 mg/kg bw/day for γ-cyhalothrin (no details available regarding the calculation)**.** Factors of 10 each for inter- and intraspecies extrapolation were used to derive the acute dietary RfD (U.S. EPA, 2017).

7 Methods of analytical determination

Analytical methods for the determination of cyhalothrin metabolites in urine, especially ClF3CA, are available.

A sensitive method was described by Schettgen et al. (2016) for the simultaneous quantification of eight metabolites of synthetic pyrethroids, including ClF3CA, in urine of the general population. The method uses acid hydrolysis of the conjugates, followed by derivatization with MTBSTFA and quantification by gas chromatography-tandem mass spectrometry (GC/MS/MS) using deuterium- and ^{13}C-labelled standards. This method has a limit of quantification of 0.01 µg/L.

8 Derivation of human biomonitoring guidance values (HBM-GV_{GenPop}) for ClF3CA in urine based on EFSA's ADI for γ-cyhalothrin

Since Health Canada applied an additional factor of 3 for the uncertainty of the database when deriving the TRV for λ-cyhalothrin, which was consistently considered unnecessary by the U.S. and European authorities, and since the assessment of the one-year oral toxicity study with dogs by the different authorities was not consistent (see footnote 3), these TRVs were not used to derive the HBM-GVs, but the EFSA/EC values were preferred. The ADIs for λ- and γ-cyhalothrin based on the endpoint "reduced body weight gain" in offspring of a multi-generation study are also supported in their order of magnitude by the acute RfDs of the U.S. EPA, which refer to acute neurotoxicity, but noting the differences of chronic vs. an acute reference value. As it is no longer possible to distinguish between the two isomer parent substances λ- and γ-cyhalothrin in human biomonitoring of metabolites, the value for γ-cyhalothrin is used as the basis for the HBM-GV derivation. Adopting the published ADI of 0.0012 mg/kg bw/day for γ-cyhalothrin (EFSA, 2014a) and taking into account the toxicokinetic basic data from the studies of Khemiri et al. (2017) and Bouchard et al. (2018), HBM-GV_{GenPop} for λ- and γ-cyhalothrin can be derived based on the excretion of the metabolite ClF3CA in urine.

The cumulative urinary excretion over the 84-h collection period, expressed as molar percentage of the orally applied cyhalothrin dose, was 21.3 ± 8.9 % in case of ClF3CA (average ± SD, n=7) (Bouchard et al., 2018; Khemiri et al., 2017) which was used to set the urinary excretion factor (Fue). The molar mass of cyhalothrin is 449.9 g/mol, that of its metabolite ClF3CA is 242.6 g/mol, thus the molar ratio of ClF3CA to cyhalothrin amounts to 0.539. The currently used body weight proportional urine volume per day (Uwp) enters the calculation with 0.03 L/kg bw/day for children and men and 0.035 L/kg bw/d for woman. The values for adults were recently determined data-based by the German HBM Commission (German HBM Commission, 2025, submitted) and replace the originally by the German HBM Commission proposed and generally used default value for adults of 0.02 L/kg bw/d.

Using a simple toxicokinetic model, the mass balance approach assuming steady-state, the following formula has to be applied: HBM-GV_{GenPop} : ADI x (molar mass ClF3CA/molar mass γ-cyhalothrin) x Fue : U_{wp}

The ADI of 0.0012 mg/kg bw/day corresponds to the following HBM-GV_{GenPop} for ClF3CA in urine:

ClF3CA, HBM-GV_{GenPop} – children and men: 0.0012 mg/kg bw/day x 0.539 x 0.213 : 0.03 L/kg bw/day = 0.0046 mg/L, **→ 4.6 µg/L**

ClF3CA, HBM-GV_{GenPop} - women: 0.0012 mg/kg bw/day x 0.539 x 0.213 : 0.035 L/kg bw/day = 0.0039 mg/L, **→ 3.9 µg/L**

The HBM-GVs are preferably given in relation to volume and not in relation to creatinine, as the physiological creatinine formation in healthy adults varies according to gender and is significantly reduced with age. Creatinine formation in children is also significantly lower and, moreover, strongly age-dependent. Meat consumption also contributes as an external factor to the creatinine pool. These aspects should be considered when adjusting biomarker measurements in urine for creatinine (Carrieri et al., 2001; Commission Human Biomonitoring of the German Environment Agency, 2005; Sallsten and Barregard, 2021; Abraham et al., 2023).

Level of confidence in the derived values: As mentioned in the HBM4EU concept document to derive HBM-GVs (Apel et al., 2020), a level of confidence (low, medium or high or intermediate levels thereof) should be attributed to each HBM-GV derived. The level of confidence should reflect the uncertainties identified during the elaboration of the value and could constitute an adequate incentive to later revise values with an estimated 'lower' level of confidence. It is evaluated according to rough individual criteria by expert judgement, whereby the equal consideration of the individual assessments results in the overall confidence level.

For (λ- and γ-) cyhalothrin the toxicological data base is extensive. Evaluations were performed by several competent authorities within the process of approval of λ- and γ-cyhalothrin for use as biocide or pesticide with some selected key studies being used for the TRV derivation. However, as an ED assessment is ongoing, the original toxicological data are not fully available to the authors of this report here, and the ADIs for λ- and γ-Cyhalothrin were established on the basis of a study conducted with cyhalothrin with the addition of additional uncertainty

factors, the level of confidence in the toxicity data is rated as only "**medium**". An improved data situation with regard to these aforementioned points could lead to an improved level of confidence.

Sensitive analytical methods for the determination of cyhalothrin metabolites in urine are available allowing for the determination of the metabolite ClF3CA with a limit of detection (LoQ) in the order of 0.01 µg/L (Schettgen et al., 2016). The toxicokinetic parameters (especially, the fraction of metabolite excreted in urine) used for the calculation of the proposed HBM-GV_{GenPop} were derived from a well-performed controlled human study published in detail. However, it must be noted that the fractional urinary excretion of the metabolite ClF3CA showed considerable interindividual variability (Bouchard et al., 2018; Khemiri et al., 2017); a lower fractional excretion of ClF3CA observed in some volunteers would lead to a lower HBM-GV[7]. On the other hand, a higher fractional excretion (about 59 %) as reported in an unpublished study (EC, 2013b; JMPR, 2007) would lead to a higher HBM-GV. Therefore, the level of confidence in the toxicokinetic data is rated as "**medium**".

We know that the urine volume of adults per day has increased over time, so that the 24h bodyweight-related urine volume used to calculate the HBM-GVs has beenadjusted based on a sound data base. Thus, the level of confidence in the calculation basis is rated as "**medium-high**".

Overall, the level of confidence in the proposed HBM-GV for cyhalothrin is rated "**medium**".

8.1 Discussion

The proposed HBM-GV_{GenPop} is based on the ADI of EFSA (2014a)/ EC (2021b) for γ-cyhalothrin, which is lower than the corresponding ADI for λ-cyhalothrin (see Table II.5). This represents a conservative approach which seems justified as the determination of the metabolite ClF3CA does not allow to discriminate between the exposure of either cyhalothrin. This is also the explanation why the HBM-GVs for λ-cyhalothrin provisionally derived under HBM4EU (Tarrazona et al., 2022) turn out to be higher (HBM-$GV_{GenPop\text{ - children}}$: 9 µg ClF3CA/L urine; HBM-$GV_{GenPop\text{ - adults}}$: 14 µg ClF3CA/L urine) than the values put to the vote here.

The ADI of EFSA/EC for γ-cyhalothrin is based on the NOAEL for effects in offspring in a multi-generation study, this NOAEL refers to a decrease in body weight gain. However, it must be noted that the derivation of an ADI or RfD based on neurotoxic effects would lead to a very similar result when taking into account the same extrapolation factors as used for the derivation of the ADI of EFSA/EC (see table II.5). Thus, the ADI of EFSA/EC and the HBM-GVs based on this ADI also take into account the protection against neurotoxic effects of γ-cyhalothrin in the general population.

It must be further taken into account that ClF3CA is not a metabolite strictly specific for cyhalothrin but is also produced and excreted as a metabolite of the pyrethroids bifenthrin and tefluthrin. However, at the time of its approval as biocide, bifenthrin had only one registered product which was a wood preservative authorized in Austria, Denmark, France, Germany, Sweden and the Netherlands. Therefore, the exposure of the EU population to bifenthrin from its biocidal use can be considered very low and further decreases in the EU because bifenthrin is no longer approved within the EU as pesticide since 2019 and as biocide since January 2023. However, bifenthrin could be present in food products imported into the EU. Therefore, it is suggested that in individual cases in which a higher concentration of ClF3CA in urine (close to or exceeding the proposed HBM-GV) is measured, further investigations may be performed, including the determination of the bifenthrin-metabolite MPBA, to differentiate between exposure to cyhalothrin and bifenthrin. The other synthetic pyrethroid containing the ClF3CA moiety is tefluthrin (2,3,5,6-tetrafluoro-4-methylbenzyl (1RS,3RS)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane¬carboxylate) (CAS-No. 79538-32-2, EC-No. 616-699-6). Principally, human exposure to tefluthrin can therefore be expected to lead to the excretion of ClF3CA in urine. However, tefluthrin is approved in the European Union only as insecticide in capsule suspension for seed coating performed in professional seed treatment facilities (EC, 2021a) and in temporary emergency approvals for the laying of potatoes in the field for

[7] Bouchard et al. (2018) calculated a BRV for the excretion of ClF3CA in urine, using the set of parametric values that generated the most protective value based on data from an individual volunteer in their toxicokinetic study. The derived chronic BRV of 0.08 nmol ClF3CA/(kg bw x d) per 0.001 mg λ-cyhalothrin/(kg bw x d) corresponds to a value of 0.2 nmol ClF3CA/(kg bw x d) per 0.0025 mg/(kg bw x d) (the ADI for λ-cyhalothrin)

treatment against wireworms and click beetle larvae (Proplanta, 2021). No relevant consumer exposure is expected from these applications. Expiry date of approval is 31. May 2027.

Due to the short half-life of elimination, the reliability of measurement results from spot urine samples compared to 24-hour urine could be questioned. Aylward et al. (2017) examined data sets for analytes with relatively short elimination half-lives from a total of 16 individuals with collection and measurement of serial urine voids over multiple days and showed, that spot sample concentrations at the 95th percentile were similar to or slightly higher than the 95th percentile of the distribution of all 24-h composite void concentrations, but tended to overestimate the maximum of the multiday concentration averages for most analytes (usually by less than a factor of 2). The GM of the spot sample distribution generally under-predicted the corresponding GM of the distribution of 24-h composite samples. These observations can assist in the interpretation of population distributions of spot samples for frequently detected analytes with relatively short elimination half-lives.

The derived HBM-GVs represent the concentration of ClF3CA in human urine up to which – for single substance evaluation – a risk for adverse health effects is not anticipated.

However, when assessing HBM results, it must be taken into account that exposure to mixtures of substances is usually present, so that the additional use of a MAF (mixture assessment factor/mixture allocation factor) or another suitable methodology should be considered for further assessments. At least structurally related substances or substances that display the same effect targets should be considered together for the next review and refinement of the HBM-GV.

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Part III

Update for the **Chromium (VI)** exposure equivalents for cancer risk – occupational assessment values

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Responsible author(s)	Selma Mahiout, TTL, selma.mahiout@ttl.fi Tiina Santonen, TTL, tiina.santonen@ttl.fi
Co-authors	Nancy Hopf (Unisanté)/ nancy.hopf@unisante.ch Robert Pasanen-Kase (SECO)/ robert.pasanen-kase@seco.admin.ch Jonna Weisell-Laitinen, TTL/ Jonna.Weisell-Laitinen@ttl.fi Paul T.J. Scheepers, RU/ paul.scheepers@ru.nl
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External reviewers	Experts of Belgian NH, French NH, German NH, Swedish NH, Polish NH, Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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1 PU = Public

PP = Restricted to other programme participants (including the Commission Services)

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Abstract

In addition to HBM-GVs, in HBM4EU an analogous procedure was applied to derive HBM Exposure Equivalents for Cancer Risk (HBM-EECR) for non-threshold carcinogens, for which it is not possible to identify a safe level of exposure. This derivation approach allows a defined risk-based assessment.

Since the derivation of the previous HBM-EECR, the European Commission's (EC) Advisory Committee on Safety and Health at Work (ACSH) issued an opinion on limit value setting for non-threshold carcinogens using a risk-based approach (ACSH 2022). In its opinion, the ACSH established that in the future, limit values for non-threshold carcinogens should be set in between the "upper risk level" of 4:1000 predicted cancer cases and the "lower risk level" of 4:100 000 (terminology as defined by the ACSH). The assumption is that exposure occurs over 8 hours per day, 5 days a week and 40 years of working life. Therefore, the HBM-EECR of 8 µg Cr/g crea, derived within HBM4EU, which corresponds to excess lifetime lung cancer risk of 20:1000, was considered too high, and the HBM-EECR should be updated within PARC. Here, HBM-EECRs were derived, based on the evaluated lung cancer risk level of 4:1000, corresponding to atmospheric levels of 1 µg Cr(VI)/m^3 (SCOEL 2017): 2 µg Cr/g crea for workers exposed to highly soluble CrVI compounds and 1 µg Cr/g crea for workers exposed to Cr(VI) oxides formed under hot conditions. At lower occupational exposure levels, the resulting urinary Cr levels may not exceed the reference limit values set based on the general (non-occupationally exposed) population's 95th percentiles. Therefore, based on the currently available data, controlling occupational exposures below the lung cancer risk levels of 4:10 000 or 4:100 000 would in practice mean that the general population reference values should not be exceeded.

Key Words

Human biomonitoring, HBM, HBM-EECR, metals, chromium VI, workers, biomarker of exposure

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Authors and Acknowledgements

Authors

Selma Mahiout, TTL, selma.mahiout@ttl.fi

Tiina Santonen, TTL, tiina.santonen@ttl.fi

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Background

Hexavalent chromium [Cr(VI)] compounds are known occupational carcinogens. There is strong evidence for their lung carcinogenicity in humans, for an increased risk for nose and nasal sinus cancer, and in addition Cr(VI) is suspected of causing stomach and laryngeal cancer (den Braver-Sewradj et al. 2021; IARC 2012). Non-cancer effects of Cr(VI) in humans include skin ulcers, nasal septum ulcerations and perforation, chronic lung diseases (asthma, pulmonary fibrosis and COPD) and allergy (rhinitis and contact dermatitis) (Hessel et al. 2021). In experimental animals, Cr(VI) has induced reproductive and developmental toxicity (ECHA 2015), but it is currently not clear if this applies also to humans.

Under the HBM4EU project (www.hbm4eu.eu), jointly agreed Human Biomonitoring Guidance Values (HBM-GVs) were derived for the HBM4EU priority substances (Apel et al. 2020). The HBM-GVs are intended to be directly compared with HBM data, thus making them a valuable tool for harmonised health risk assessment. As a concept, HBM-GVs are similar to several other existing limit- or guidance values, such as the Occupational Biomonitoring Levels (OBL[8]) by the OECD (OECD 2022) and biological limit values (BLVs) by the ECHA Risk assessment committee (RAC[9]), and previously Scientific Committee on Occupational Exposure Limits (SCOEL[10]).

In addition to HBM-GVs, in HBM4EU an analogous procedure was applied to derive HBM Exposure Equivalents for Cancer Risk (HBM-EECR) for non-threshold carcinogens, for which it is not possible to identify a safe level of exposure. This derivation approach allows a defined risk-based assessment.

For Cr(VI), an HBM-EECR was derived within HBM4EU for workers of the chrome-plating sector by Sissoko et al., available in (Apel et al. 2022). The HBM-EECR of 8 µg Cr/g creatinine (crea) was based to correspond with the current (then upcoming) European 8-h binding occupational exposure limit value (BOELV) of 5 µg Cr(VI)/m^3 (in force from January 17 2025, Directive 2017/239844 amending Directive 2004/37/EC). This 8-h BOELV has been evaluated to correspond to a predicted excess lifetime lung cancer risk of 20 cases per 1000 employees (20:1000) (SCOEL 2017), exceeding the desired protection levels of several EU Member States and associated countries.

In workplaces, exposure levels to carcinogens should be reduced to as low as technically possible. However, for OEL setting, a limit must be selected. Up until recently, there has not been a consensus in the EU on occupational cancer risk levels that can be considered acceptable.

Since the derivation of the previous HBM-EECR, the European Commission's (EC) Advisory Committee on Safety and Health at Work (ACSH) issued an opinion on limit value setting for non-threshold carcinogens using a risk-based approach (ACSH 2022). In its opinion, the ACSH established that in the future, limit values for non-threshold carcinogens should be set in between the "upper risk level" of 4:1000 predicted cancer cases and the "lower risk level" of 4:100 000 (terminology as defined by the ACSH). The assumption is that exposure occurs over 8 hours per day, 5 days a week and 40 years of working life.

Therefore, the HBM-EECR of 8 µg Cr/g crea, derived within HBM4EU, which corresponds to excess lifetime lung cancer risk of 20:1000, was considered too high, and the HBM-EECR should be updated within PARC (this document).

Results

HBM-EECRWorker-HighlySolubleCr(VI)compounds(4x10^{-3}): 2 µg Cr/g creatinine (2.7 µg Cr/L)

HBM-EECRWorker-Cr(VI)OxidesFormedInHotConditions (4 x 10^{-3}): 1 µg Cr/g crea (1.4 µg Cr/L urine)

[8] Occupational Biomonitoring: https://www.oecd.org/chemicalsafety/risk-assessment/occupational-biomonitoring.htm
[9] Occupational exposure limits substance evaluations: https://echa.europa.eu/oels-activity-list
[10] Recommendations of the SCOEL: https://echa.europa.eu/recommendations-of-the-scoel

Abbreviations and Acronyms

ACSH	Advisory Committee on Safety and Health at Work
BOELV	Binding Occupational Exposure Limit Value
Crea	creatinine
Cr(VI)	Hexavalent chromium
HBM-EECR	HBM Exposure Equivalents for Cancer Risk
RPE	Respiratory Protective Equipment
SCOEL	Scientific Committee on Occupational Exposure Limits

1 Introduction - Rationale for the update of HBM-EECR

In addition to the high estimated cancer risk level of 20:1000 at the current OEL level of 5 µg Cr(VI)/m^3, also increased effect biomarker levels (namely related to genotoxicity and oxidative stress) have been observed at the currently measured Cr(VI) exposure levels, particularly in chrome painters and electrolytic bath platers (Tavares et al. 2022). Their median levels for the inhalable fraction were 5.61 µg Cr(VI)/m^3 for chrome painters and 0.43 µg Cr(VI)/m^3 for electrolytic bath platers, and the respective 95th percentiles were 154 µg/m^3 and 5.13 µg/m^3 (Santonen et al. 2022). This further supports the view that the current Cr(VI) exposure may represent a health risk, highlighting the need to lower the current occupational assessment values.

The basis for the PARC HBM-EECR for Cr(VI) are the cancer risk levels that will be associated with limit values for non-threshold carcinogens according to the ACSH opinion, ranging from 4:1000 (temporarily tolerable) to 4:10 000 and 4:100 000 (acceptable). The risk level of 4:1000, corresponding to the Cr(VI) air concentration of 1 µg Cr(VI)/m^3 (SCOEL 2017), was chosen as the basis for the derivation of the HBM-EECR. As already mentioned, the current European 8-h OEL is higher at 5 µg Cr(VI)/m^3, but it is likely to receive an update at some point, following the ACSH opinion.

It should be noted that the ACSH concluded that if an initial OEL value is set at a risk level above 4:10 000, it should be a priority aim to replace it by a more protective OEL that will apply after a transition period (ACSH 2022). Moreover, they stated that an updating/revising period should be introduced when it is not possible to yet agree on a more protective OEL and a transition period. However, deriving an HBM-EECR equivalent to the lower air values of 0.1 or 0.01 µg Cr/m^3, corresponding to lung cancer risk levels of 4:10 000 or 4:100 000, respectively, is not possible based on the currently available data. The resulting HBM-EECR urinary levels would be so low that they could not be reliably distinguished from the background urinary Cr levels in the general population (see Table III.1 and Discussion).

2 Derivation of an updated HBM-EECR for Cr(VI)

The previous HBM4EU deliverable, presenting the derivation of the HBM-EECR of 8 µg Cr/g crea, includes an extensive summary of the literature (Sissoko et al., available in (Apel et al. 2022)), which is not repeated here. Please see it for information on Cr and Cr(VI), the identification of Cr(VI), its physico-chemical properties, uses of Cr(VI) compounds, existing EU classification and regulation on Cr(VI), human exposure to Cr(VI), detailed toxicological profile of Cr(VI) including toxicokinetics and health effects, and a description of the existing toxicity reference values for Cr(VI) compounds for occupationally exposed workers. In addition, information on HBM parameters of Cr(VI) and related analytical methods is provided there.

2.1 Derivation method

Here, the derivation of the HBM-EECR is performed using the same approach that was used in HBM4EU, i.e. employing correlations between measured Cr(VI) air concentrations and urinary total chromium levels (Apel et al. 2022). **Table III.1** presents the data and regression formulas used here, based on measurements from chrome platers and welders.

Two different values are derived, separately for workers exposed to highly soluble Cr(VI) compounds, (e.g. in chromium plating activities) and workers exposed to Cr(VI) oxides formed under hot conditions (e.g. in welding or manufacturing of stainless steel). This is due to Cr(VI) occurring in the workplace atmospheres in a variety of physical and chemical forms, and thus also the Cr(VI) exposure patterns being different.

Chrome plating involves exposure to highly soluble Cr(VI) compounds, such as chromic acid (chromium trioxide) in an aerosol form. These particles have high bioavailability; they are absorbed easily from the lungs, reduced to Cr(III) and rapidly excreted to the urine (ATSDR 2012). In contrast, Cr(VI) that is formed in welding (of stainless steel), is emitted as welding fumes. The chemistry is more complex and will result in complex mixtures of more scarcely water-soluble to water insoluble ultrafine chromium oxide particles, with toxicity related to their small particle size and low solubility in the airways. This translates to long–very long retention in the lungs, much lower bioavailability and consequently slower urinary excretion (Antonini et al. 2010; Scheepers et al. 2008).

The difference in the kinetics is likely reflected in the published regression formulas for plating and welding activities (Viegas et al. 2022). Because of this, two separate HBM-EECRs are warranted (Santonen et al. 2022). In the first derivation by Sissoko et al., available in (Apel et al. 2022), it was likewise considered that the then derived HBM-EECR was applicable only for workers of the chrome-plating sector. They also calculated a separate urinary Cr concentration for welders, but did not derive an HBM-EECR for them. This was presumably because the publication providing the equation for welders had not been published yet, although was available to the authors as *in preparation*.

2.2 Calculation of the HBM-EECR$_{worker}$

Sissoko et al. summarised in their report the available studies reporting measurements of atmospheric levels of Cr(VI) in relation to urine levels of total Cr (Table XIV.43 and Annex of (Apel et al. 2022)). Since then, also a new study by (Viegas et al. 2022) has been published, including separate equations for bath plating workers and welders (included as "in preparation" also by Sissoko et al., available in (Apel et al. 2022)).

Here, for plating we are using two of the same equations from (Chen et al. 2002) that were used in the HBM4EU work by Sissoko et al., available in (Apel et al. 2022). In addition, we have used the new and only equation available for welding (Viegas et al. 2022). Both key studies are briefly described in Table III.1.

As mentioned by Sissoko et al., available in (Apel et al. 2022), correct sampling times are an important part of the biological monitoring strategy in occupationally exposed adults, and are based on the toxicokinetic properties of the biomarker of exposure in question. Concerning the sampling of urinary Cr, the best time for sampling is at end of shift and end of work week, reflecting exposure from preceding days together with long-term exposure.

As mentioned before, the starting point as the reference atmospheric concentration for the derivation of the HBM-EECR$_{Worker}$ was 1 µg Cr(VI)/m^3, which corresponds to an excess lifetime lung cancer risk of 4 cases per 1000

workers exposed for 8 hours a day for 40 years, as assessed earlier by SCOEL (SCOEL 2017) (see Table III.1). From there, also equivalents for the cancer risk levels of 4:10 000 and 4:100 000 were calculated.

For highly soluble Cr(VI) compounds, the calculated levels of urinary Cr, based on equations from two studies on platers (Chen et al. 2002; Viegas et al. 2022), are 1.65 µg Cr/g crea and 1.98 µg Cr/g crea. The value of 2 µg Cr/g crea is obtained by averaging the two urinary concentrations (resulting in 1.8 µg Cr/g crea) and rounding it up. For Cr(VI) oxides formed under hot conditions, the calculated level of urinary Cr, based on the only available equation for welders (Viegas et al. 2022), is 1.2 µg Cr/g crea. By rouding down, the value of 1 µg Cr/g crea is obtained. Conversions to the unit µg Cr/L urine were performed as described in the Supplementary Table III.5 in (Santonen et al. 2023). In practice, by multiplying the µg Cr/g crea result by a mean urinary creatinine concentration (in g/L). The mean urinary creatinine concentration used was 1.36 g of creatinine/L, based on (Cocker et al. 2011).

Upper risk level for workers exposed to highly soluble Cr(VI) compounds, e.g. in chromium plating activities:

$HBM\text{-}EECR_{WorkerHighlySolubleCrVICompounds(4 \times 10^{-3})}$: 2 µg Cr/g crea (2.7 µg Cr/L urine)

Upper risk level for workers exposed to Cr(VI) oxides formed under hot conditions, e.g. in welding or manufacturing of stainless steel:

$HBM\text{-}EECR_{WorkerCr(VI)OxidesFormedInHotConditions\ (4 \times 10^{-3})}$: 1 µg Cr/g crea (1.4 µg Cr/L urine)

These upper risk values apply to exposures to Cr(VI) in the chrome-plating sector and the welding sector, and sectors with very similar exposures, such as production of stainless steel. They correspond to an excess lifetime lung cancer risk of 4 cases per 1000 workers, exposed 8 hours a day for 40 years to an atmospheric concentration of 1 µg Cr(VI)/m^3.

According to the ACSH opinion, if a set limit value is associated with an excess cancer risk level above 4:10 000, priority action is needed to review the limit value in the future to be replaced by a more protective value (see Discussion). In addition, irrespective of limit values, at workplaces where genotoxic exposures can occur (such as Cr(VI) exposure), exposure should be avoided or minimised, aiming to controlled exposure levels corresponding to a cancer risk level of 4 cases per 100 000 workers.

Table III.1: Summary of the data and regression formulas used for the derivation of the HBM-EECR values, including the calculated internal exposure levels corresponding to the external exposure levels corresponding to lung cancer risk levels of 4:1000 – 4:100 000.

Ref.	Work covered	Sample collection	Atmospheric and urinary concentrations measured	Further information	Equation linking atmospheric Cr(VI) and urinary total Cr	Calculation of total urinary Cr (µg/g crea) for 8-h inhalation exposure and the corresponding lung cancer risk [a]		
						1 µg Cr(VI)/m³, cancer risk 4:1000	0.1 µg Cr(VI)/m³, cancer risk 4:10 000 [b]	0.01 µg Cr(VI)/m³, cancer risk 4:100 000 [b]
(Chen et al. 2002)	Decorative and hard-surface Cr plating	Atmospheric samples of inhalable Cr(VI) aerosols were taken with personal samplers over one whole working day (last day of the work week). Workers did not use RPE but were requested to wear an apron and gloves for the sampling work week. Urine samples (one sample from each exposed worker) was collected at end of the work week, at the end of shift.	Air (soluble Cr^{+6}; µg/m³): Decorative plating AM 1.91, range 0.340–14.0. Hard-surface plating AM 25.2, range 4.40–96.0. Urine (Cr; µg/g crea): Decorative plating AM 3.43, range 0.633–29.2. Hard-surface plating AM 46.2, range 7.73–187.	n=57 (Decorative plating n=27, Hard-surface plating n=30); goodness of fit r^2=0.87	$[Cr_{urine}]$ (µg/g crea) = 1.86 $[Cr(VI)_{air}]$ (µg/m³) - 0.21	1.65 µg Cr/g crea	-0.02 µg Cr/g crea	-0.19 µg Cr/g crea
(Viegas et al. 2022)	Cr bath plating	Atmospheric samples of the inhalable and respirable fractions of Cr(VI) were taken with personal samplers over a representative period of a work shift. Workers included in the calculation of correlations did not use RPE. Urine samples were collected at the beginning and end of the same work week (pre- and post-shift samples).	Air (inhalable Cr(VI), outside RPE, µg/m³, n=57): GM 0.3, range <LOQ–9.1. Urine (total U-Cr µg/g crea; post-shift, n=90): GM 1.2, range 0.1–13.6.	Correlations from sub-group not wearing RPE, n=42; Spearman correlation coefficient r_s=0.858; r^2=0.679	$[Cr_{urine}]$ (µg/g crea) = 0.742 + 1.235 $[Cr(VI)_{air}]$ (µg/m³)	1.98 µg Cr/g crea	0.87 µg Cr/g crea	0.75 µg Cr/g crea
(Viegas et al. 2022)	Welding	Atmospheric samples of Cr(VI) were taken with personal samplers over a representative period of a work shift. Workers included in the calculation of correlations did not use RPE. Urine samples were collected at the beginning and end of the same work week (pre- and post-shift samples).	Air (inhalable Cr(VI) outside RPE, µg/m³, n=107): GM 0.5, range <LOQ–40.4. Urine (total U-Cr, µg/g crea; post-shift, n=189): GM 0.7, range 0.1–5.8.	Correlations from sub-group not wearing RPE, n=46; r_s=0.515; r^2=0.324	$[Cr_{urine}]$ (µg/g crea) = 0.647 + 0.541 $[Cr(VI)_{air}]$ (µg/m³)	1.19 µg Cr/g crea	0.70 µg Cr/g crea	0.65 µg Cr/g crea

crea=creatinine, RPE=respiratory protective equipment, AM=arithmetic mean, GM=geometric mean.

[a] Lung cancer risks evaluated by (SCOEL 2017), except for the level 4:100 000, for which they did not include an evaluation and was extrapolated here based on the two available risk level evaluations.

[b] In studies published within the past ten years, median urinary Cr levels in the European general population have been in the range of 0.1–0.4 µg Cr/g crea, P95 values 0.4–1.4 µg Cr/g crea (Aprea et al., 2018; Morton et al., 2014; Santonen et al., 2022).).

2.3 HBM-EECR$_{worker}$ level of confidence

As described in (Apel et al. 2020), a level of confidence (low, medium, high or their intermediates) should be attributed to the calculated values. It should reflect the identified uncertainties related to the nature and quality of the data, the choice of the critical effect and the mode of action, the key study, the choice of the critical dose (POD) and the extrapolations across and within species. The level of confidence proposed here is not determined according to an established methodology, but relies on expert judgment.

Related to both HBM-EECRs:

- The level of confidence in the nature and quality of the toxicological data is considered medium. The toxicological profile of Cr(VI) is well documented in numerous studies, also in humans. However, there are no epidemiological data on the direct relationship between (urinary) biomarker concentrations in humans and the adverse health effects.
- The level of confidence in the choice of the critical effect and the mode of action is considered high. There are several risk assessments made by different expert groups, and the evidence of the carcinogenic effects and mechanisms is robust and well documented. Lung cancer has been identified as the critical endpoint.
- The level of confidence in the OEL for atmospheric Cr concentrations proposed by SCOEL is considered medium–high (SCOEL 2017). It was made by an expert group and is rather recent. Within the past ~10 years, also other expert groups have concluded on the same or very similar cancer risk estimates for lung cancer (AGS 2014; DECOS 2016; ECHA 2013; NIOSH).

Selection of key studies for identification of the relationship between atmospheric concentrations of Cr(VI) and urinary Cr concentrations:

For **plating activities**, where exposure is to highly soluble Cr(VI), there are several studies available providing measured data on correlations between atmospheric Cr(VI) and urinary Cr levels (described in detail by Sissoko et al., available in (Apel et al. 2022)). The most recent data from the HBM4EU chromate study (Viegas et al. 2022) are very well in line with the most recent earlier studies, demonstrating that the correlation data is robust. The confidence in the correlation data on plating activities is considered medium. Therefore, the overall confidence in the HBM-EECR$_{WorkerHiglySolubleCrVICompounds}$ of 2 ug Cr/g crea is considered **medium**.

This applies particularly for the cancer risk level of 4:1000, which corresponds to atmospheric concentrations of 1 µg Cr(VI)/m^3, as the regression formula used is based on measured data. However, extrapolating from there to the much lower cancer risk levels of 4:10 000 and 4:100 000 brings added uncertainty, as visible in Table III.1. In addition, deriving an HBM-EECR for the air values of 0.1 or 0.01 µg Cr/m^3, corresponding to cancer risk levels of 4:10 000 or 4:100 000, respectively, would not be possible because the corresponding urinary levels could not be reliably distinguished from the background urinary Cr levels in the general population (see Table III.1 and Discussion).

For **welding**, where the exposure is to completely different Cr(VI) compounds, the data on correlation is considerably less robust and includes significant uncertainties. Only one dataset is available (Viegas et al. 2022), with only a moderate correlation coefficient for the regression formula. The confidence in the correlation data on welding activities is considered low. Therefore, the overall confidence in the HBM-EECR$_{Cr(VI)OxidesFormedInHotConditions\ (4\ x\ 10^{-3})}$ of 1 µg Cr/g crea is considered **low–medium**. However, it provides a clear indication that for these Cr(VI) compounds, an HBM-EECR of 2 µg Cr/g crea would not be conservative enough to indicate exposure to Cr(VI) levels exceeding the atmospheric limit value of 1 µg Cr/m^3 (and estimated cancer risk level of 4:1000). Therefore, this separate HBM-EECR is considered justified although the confidence level is lower (Santonen et al. 2022).

Urinary Cr concentration reflects exposure via all relevant exposure routes (inhalation and ingestion), and exposure to both carcinogenic Cr(VI) and non-carcinogenic Cr(III). This is however likely reflected in the regression formulas, as dermal contamination correlated strongly with Cr(VI) air levels outside of respiratory protective equipment (RPE) in the HBM4EU chromate study (Santonen et al. 2022). The use of these regression equations assumes that this correlation between air and dermal levels is similar in these types of jobs.

No RPE was worn by the workers included in the studies producing the regression formulas used here, but in (Chen et al. 2002) the plating workers were requested to wear an apron and gloves for the sampling work week. It was not reported how well this was accomplished.

3 Discussion

Cr(VI) is a known lung carcinogen (den Braver-Sewradj et al. 2021; IARC 2012). However, there are no epidemiological data that would allow a quantitative risk assessment based on a direct dose-response relationship between internal exposure (e.g. urinary Cr) and adverse health effects, as described as the preferred approach in (Apel et al. 2020). Therefore, as the next recommended approach, the derivation of HBM-EECRs for workers was based on an occupational exposure limit for external exposure, derived previously by SCOEL (SCOEL 2017).

Urinary Cr was selected as the relevant biomarker, although it reflects total Cr exposure, and is therefore not specific to internal exposure to Cr(VI). This was because the majority of the available regression formulas show good correlation between atmospheric Cr(VI) and urinary total Cr. Also, correlation to other matrices has been studied. Recently, for example, the HBM4EU chromates study examined the usefulness of measuring total Cr in red blood cells or plasma, or Cr(III) and Cr(VI) levels in exhaled breath condensate, in the assessment of occupational Cr(VI) exposure (Leese et al. 2023; Ndaw et al. 2022). At least so far, the data are scarce and the resulting regression analyses linking internal and external Cr exposure provide less robust correlations than those linking urinary Cr and atmospheric Cr(VI) concentrations.

Within HBM4EU, an HBM-EECR of 8 µg Cr/g crea was derived for workers of the chrome-plating sector (Sissoko et al., available in (Apel et al. 2022)). This HBM-EECR was based to correspond with the current European 8-h OEL of 5 µg Cr(VI)/m^3, and corresponds to a predicted excess lifetime lung cancer risk of 20:1000 (SCOEL 2017). Since then, the EC's ACSH has established that in the future, limit values for non-threshold carcinogens should be set in between the risk levels of 4:1000–4:100 000 (ACSH 2022).

Therefore, here, revised HBM-EECRs were derived, based on the evaluated lung cancer risk level of 4:1000, corresponding to atmospheric levels of 1 µg Cr(VI)/m^3 (SCOEL 2017): **2 µg Cr/g crea for workers exposed to highly soluble CrVI compounds and 1 µg Cr/g crea for workers exposed to Cr(VI) oxides formed under hot conditions**. The same methodology was employed as by Sissoko et al. earlier, namely published regression formulas that correlate measured atmospheric Cr(VI) concentrations and urinary total Cr concentrations (Chen et al. 2002; Viegas et al. 2022).

The level of confidence in the HBM-EECR$_{\text{WorkerHighlySolubleCrVICompounds}}$ of 2 ug Cr/g crea is considered medium. The Confidence in the HBM-EECR$_{\text{WorkerCr(VI)OxidesFormedInHotConditions (4 x 10-3)}}$ of 1 µg Cr/g crea is considered low–medium. It is however considered justified, because the available data indicates that for Cr(VI) oxides formed under hot conditions, an HBM-EECR of 2 µg Cr/g crea would not be conservative enough.

In studies published within the past ten years, median urinary Cr levels in the European general population have been in the range of 0.1–0.4 µg Cr/g crea and P95 values in the range of 0.4–1.4 µg Cr/g crea (Aprea et al. 2018; Morton et al. 2014; Santonen et al. 2022).

The cancer risk level of 4:1000 is still high, although considered by the ACSH the upper risk level associated with a limit value (that should be lowered in the future) (ACSH 2022). In workplaces however, exposure to carcinogens should be as low as technically possible. For setting of national limit values, policy makers may wish to consider setting a lower value for Cr(VI) exposure by applying the precautionary principle.

It should however be noted that at lower occupational exposure levels, the resulting urinary Cr levels may not exceed the reference limit values set based on the general (non-occupationally exposed) population's 95th percentiles (see Table III.1). Therefore, based on the currently available data, controlling occupational exposures below the lung cancer risk levels of 4:10 000 or 4:100 000 would in practice mean that the general population reference values should not be exceeded. (Reference limits may vary between populations and regions and must be based on the data obtained from the same region/country.)

In addition to the uncertainties discussed under the chapter "HBM-EECR$_{\text{worker}}$ level of confidence", it should also be noted that welding fumes include also other carcinogenic agents than Cr(VI), namely nickel. Mixture effects could not be considered in the setting of this HBM-EECR.

Options to assess combined mixture risks with different micronuclei and Comet assay methods have recently been investigated by an OECD activity under the Working Parties on Exposure and Hazard Assessments (WPEA & WPHA). Their evaluation, soon to be published, suggests that an induction of micronuclei exceeding a factor 2 in

the Human Lymphocyte Cytokinesis Block Micronucleus Assay may be linked with an increased risk of several cancer and non-cancer diseases (Fenech et al. 2021; OECD 2025 in prep.). This effect-biomarker could possibly be used to indicate co- exposures of several genotoxic carcinogens. Related to Cr(VI) exposure, the findings of (Tavares et al. 2022) indicate that in processes like bath plating, this level can be exceeded.

The single substance derived values of 2 µg Cr/g crea for workers exposed to highly soluble CrVI compounds and 1 µg Cr/g crea for workers exposed to Cr(VI) oxides formed under hot conditions, corresponding to atmospheric concentrations of 1 µg Cr(VI)/m^3, are well in line with recent values set elsewhere. In France, ANSES has proposed a BLV of 2.5 µg/L (1.8 µg/g crea) for biomonitoring of Cr(VI) compounds[11] (ANSES 2017). For atmospheric Cr(VI) compounds and/or chromic acid and chromates, the current 8h-OELs are set at 1 µg/m^3 in France, Denmark, Norway, Germany (for inhalable fraction) and the Netherlands (for soluble compounds) (IFA 2023). In the USA, NIOSH has set a lower value of 0.2 µg/m^3 for Cr(VI) compounds, chromic acid and chromates. New Zealand has the lowest 8-h OEL value for Cr(VI) compounds at of 0.02 µg/m^3.

In the occupationally exposed population (covering e.g. platers and welders), we recently reported a decreasing trend in urinary Cr concentrations in Finland over the past 40 years (Mahiout et al. 2022). A similar decreasing trend was observed also in Belgium (Verdonck et al. 2021). In addition, the Finnish data as well as the more recent data from the HBM4EU chromate study showed that many workplaces appear to already be able to achieve workers' urinary Cr levels corresponding to air concentrations well below the current OEL limit value of 5 µg Cr(VI)/m^3; in the HBM4EU chromate study, the majority of urinary Cr concentrations were <2 µg Cr/g crea (Santonen et al. 2022). Therefore, the atmospheric concentrations corresponding to the derived HBM-EECRs should be achievable in workplaces when adequate risk management measures are established.

[11] This value only applies to exposures to CrVI in the chrome-plating sector

4 Annex: Derivation of HBM-EECRs: key data

Table III.2: Derivation of HBM-EECR for Cr(VI), highly soluble Cr(VI) compounds (e.g. in chromium plating activities). Modified from Sissoko et al., available in (Apel et al. 2022)

Derivation of HBM-EECR		
Substance	**Cr(VI): highly soluble Cr(VI) compounds (e.g. in chromium plating activities)**	
Parameter	**Value / Descriptor**	**Comments**
HBM-EECR$_{Worker}$ and Status		
HBM-EECR$_{Worker-HighlySolubleCr(VI)compounds(4x10^{-3})}$	2 µg Cr/g creatinine (2.7 µg Cr/L)	Numerical value for HBM Exposure Equivalent for additional lifetime lung Cancer Risk (4 x 10^{-3}) for urinary Chromium, concerning the occupational population exposed to highly soluble Cr(VI) compounds (e.g. in chromium plating activities).
Level of confidence	Medium	See page 95: HBM-EECRworker level of confidence. Reflects the reliability of the proposed HBM-EECR. The level of confidence takes into account the various uncertainties underlying the derivation of the HBM-EECR and relies on an expert judgement.
Year of issue	2025	
Status	Agreed on within PARC	According to the occupational exposure level associated with the estimated lung cancer risk level 4:1000, which is below the current EU 8-h BOELV.
General Information		
CAS No	Depending on the compound	
IUPAC name	Depending on the compound	
Molar mass (g/mol)	Depending on the compound	
Biomarker(s)		
Identification	Urinary Chromium (u-Cr)	Sampling at the end of shift, end of work week
Molar mass of biomarker(s)	Depending on the compound	
Half-life of selected biomarker(s)	Three phases: 7 hours, 15–30 days, 3–5 years	Hoët (2005) in (Apel et al. 2022)
Derivation method, starting point and assessment factors		
Type of derivation method selected	Based on the relationship between the biomarker concentrations in urine and atmospheric OEL level estimated earlier by SCOEL to be associated with an excess lung cancer risk of 4:1000 (SCOEL 2017)	Option 2 for the occupational population as described in (Apel et al. 2022)

Derivation of HBM-EECR		
Substance	**Cr(VI): highly soluble Cr(VI) compounds (e.g. in chromium plating activities)**	
Parameter	**Value / Descriptor**	**Comments**
Key studies	(Chen et al. 2002; Viegas et al. 2022)	Regression formulas for correlations from these studies.
Species	Human	
Route/type of study	Inhalation, occupational field studies	
Critical endpoint	Lung cancer	
EU 8h- BOELV	5 µg/m^3	As set in the Directive 2017/239844 amending Directive 2004/37/EC. Corresponds to an excess lung cancer risk of 20:1000 (40-year exposure).
External dose for the calculation of the HBM-EECR: SCOEL estimation for OEL level associated with excess lung cancer risk of 4:1000	1 µg Cr/m^3	HBM-EECR corresponds to this atmospheric concentration
Calculation of HBM-EECR$_{WorkerHighlySolubleCr(VI)compounds(4x10^{-3})}$		
Correlations used between exposure biomarker concentrations and atmospheric levels	Chen et al. (2002), Decorative and hard-surface Cr plating: Cr_{urine}] (µg/g crea) = 1.86 [Cr(VI)$_{air}$] (µg/m^3) - 0.21 Viegas et al. (2022), Cr bath plating: [Cr_{urine}] (µg/g crea) = 0.742 + 1.235 [Cr(VI)$_{air}$] (µg/m^3)	
Result (rounded value)		
HBM-EECR$_{Worker-HighlySolubleCr(VI)compounds(4x10^{-3})}$	**2 µg Cr/g creatinine** (2.7 µg Cr/L)	

Table III.3: Derivation of HBM-EECR for Cr(VI) oxides formed under hot conditions (e.g. in welding or manufacturing of stainless steel). Modified from Sissoko et al., available in (Apel et al. 2022)

Derivation of HBM-EECR		
Substance	**Cr(VI): Cr(VI) oxides formed under hot conditions (e.g. in welding or manufacturing of stainless steel)**	
Parameter	**Value / Descriptor**	**Comments**
HBM-EECR$_{Worker}$ and Status		
HBM-EECR$_{Worker-Cr(VI)OxidesFormedInHotConditions}$ (4×10^{-3})	1 µg Cr/g crea (1.4 µg Cr/L urine)	Numerical value for HBM Exposure Equivalent for additional lifetime lung Cancer Risk (4×10^{-3}) for urinary Chromium, concerning the occupational population exposed to Cr(VI) oxides formed under hot conditions (e.g. in welding or manufacturing of stainless steel).
Level of confidence	Low-medium	See page 95: HBM-EECRworker level of confidence. Reflects the reliability of the proposed HBM-EECR. The level of confidence takes into account the various uncertainties underlying the derivation of the HBM-EECR and relies on an expert judgement.
Year of issue	2025	
Status	Agreed on within PARC	According to the occupational exposure level associated with the estimated lung cancer risk level 4:1000, which is below the current EU 8-h BOELV.
General Information		
CAS No	Depending on the compound	
IUPAC name	Depending on the compound	
Molar mass (g/mol)	Depending on the compound	
Biomarker(s)		
Identification	Urinary Chromium (u-Cr)	Sampling at the end of shift, end of work week
Molar mass of biomarker(s)	Depending on the compound	
Half-life of selected biomarker(s)	Three phases: 7 hours, 15–30 days, 3–5 years	Hoët (2005) in (Apel et al. 2022)

Derivation of HBM-EECR		
Substance	**Cr(VI): Cr(VI) oxides formed under hot conditions (e.g. in welding or manufacturing of stainless steel)**	
Parameter	**Value / Descriptor**	**Comments**
Derivation method, starting point and assessment factors		
Type of derivation method selected	Based on the relationship between the biomarker concentrations in urine and atmospheric OEL level estimated earlier by SCOEL to be associated with an excess lung cancer risk of 4:1000 (SCOEL 2017)	Option 2 for the occupational population as described in (Apel et al. 2022)
Key studies	(Viegas et al. 2022)	Regression formula for correlation from this study.
Species	Human	
Route/type of study	Inhalation, occupational field studies	
Critical endpoint	Lung cancer	
EU 8h-BOELV	5 µg/m^3	As set in the Directive 2017/239844 amending Directive 2004/37/EC. Corresponds to an excess lung cancer risk of 20:1000 (40-year exposure).
External dose for the calculation of the HBM-EECR: SCOEL estimation for OEL level associated with excess lung cancer risk of 4:1000	1 µg Cr/m^3	HBM-EECR corresponds to this atmospheric concentration
Calculation of HBM-EECR$_{WorkerCr(VI)OxidesFormedInHotConditions(4x10^{-3})}$		
Correlations used between exposure biomarker concentrations and atmospheric levels	Viegas et al. (2022), Welding: [Cr$_{urine}$] (µg/g crea) = 0.647 + 0.541 [Cr(VI)$_{air}$] (µg/m^3)	
Result (rounded value)		
HBM-EECR$_{Worker-Cr(VI)OxidesFormedInHotConditions(4x10^{-3})}$	**1 µg Cr/g creatinine** (1.4 µg Cr/L urine)	

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Part IV

Derivation of human biomonitoring guidance values for **nickel and its compounds** for workers and the general population

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Contributing Participants	Lucie Hegg, Unisante Nancy B Hopf, Unisante Petra Apel, UBA Maria Tannous and Keyvin Darney /ANSES Manca Ahačič and Lucija Perharič /NIJZ
Responsible author(s)	Jonna Weisell-Laitinen, TTL, jonna.weisell-laitinen@ttl.fi; Tiina Santonen, TTL, tiina.santonen@ttl.fi
Co-authors	Lucie Hegg, Unisante Nancy B Hopf, Unisante Petra Apel, UBA
Internal Reviewers[2]	WP4 co-leaders
External Reviewers[3]	German national hub/ sub-hub human tox Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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Abstract

In this document, human biomonitoring guidance values (HBM-GVs) for the general population and for workers are proposed for Nickel and its compounds. Toxicology data are based on previous evaluations and studies (ECHA, 2018; EFSA, 2015; EFSA, 2020; SCOEL, 2011; WHO, 2000, 2021). The general population is mainly exposed to nickel via the oral route whereas workers are typically exposed through inhalation. Nickel compounds can be divided into soluble and sparingly soluble, and their toxicokinetics differ. Soluble nickel is more readily absorbed and excreted than sparingly soluble nickel. For the general population an orientation HBM-GV range was derived from TDI 13 µg/kg bw/day recommended by EFSA (2020). This TDI value is based on reproductive toxicity as there was no evidence for carcinogenicity through oral exposure. Both the urinary mass balance approach and physiologically based pharmacokinetic (PBPK) modelling were used to estimate corresponding urine concentrations for external intake levels.

Because of the large differences between fasting und non-fasting status and differences in the form of nickel the general population is exposed to, an orientation HBM-GV range for the general population was proposed referring to the non-fasting status only. A range is specified because a defined value cannot be derived due to too many influencing variables. This indication is not to be confused with a provisional HBM-GV, which has been derived with good reason but has not yet been harmonised within PARC. The orientation range is 3-11 µg/L urine and, provided that it is known whether a subject examined in an HBM study was in a non-fasting status when the urine sample was taken, allows for an initial rough assessment as to whether there might be cause for health concern. Further investigations with additional determination of relevant influencing variables would be necessary in order to be able to make more detailed statements.

The HBM-GV for workers was based on the occupational exposure limit (OEL) value of 0.03 mg/m^3 for the inhalable fraction of nickel recommended by RAC in 2018. This value was based on nasal carcinogenicity. Available information supports a mode-of-action based threshold for carcinogenicity. Established correlations between air levels and urine nickel levels were used to derive the HBM-GV. Derivation was done separately for soluble and sparingly soluble nickel compounds. The derived HBM-GV for occupational exposure to soluble nickel compounds is 12 µg/L. The proposed value is considered to also protect from reproductive toxicity. Due to the limited bioavailability and urine excretion, no HBM-GV for sparingly soluble nickel is recommended.

Key Words

Human biomonitoring (HBM), risk assessment, metals, nickel, health-based guidance values, HBM-GV, chemical policy

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Authors and Acknowledgements

Authors

Jonna Weisell-Laitinen, TTL, Finland

Tiina Santonen, TTL, Finland

Lucie Hegg, Unisante, Switzerland

Nancy B Hopf, Unisante, Switzerland

Petra Apel, UBA, Germany

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Entity	Contributing expert
Partner ANSES (France)	Keyvin Darney, Maria Tannous
Partner NIJZ (Slovenia)	Manca Ahačič, Lucija Perharič
German NH	Sub-human tox
Partner MOH-IL (Israel)	Tamar Berman

Abbreviations and Acronyms

AF	Assessment Factor - Assessment factors are used to address the differences between experimental data and the human situation, taking into account the uncertainties in the extrapolation procedure and in the available data set.
BMD	Benchmark dose
BMDL	Benchmark dose lower bound
BMI	Body mass index
bw	body weight
CAS	Chemical Abstracts Service (division of the American Chemical Society)
CI	Confidence interval
CLP	Classification, Labelling and Packaging of substances
d	day
DNEL	Derived no-effect level
DMT1	Divalent metal transporter 1
EC	European Commission
ECHA	European Chemicals Agency
EU	European Union
FDA	US Food and Drug Administration
Fue	Urinary excretion factor
GD	Gestational day
GM	Geometric mean
GLP	Good Laboratory Practice
h	hour
HEC	Human equivalent concentration
HR	Hazard ratio
HBM4EU	European Human Biomonitoring Initiative
HBM-GV	Human Biomonitoring Guidance Value
ILO	International Labour Organization
i.p.	Intaperitoneal
LC50	Lethal concentration 50 %
LD50	Lethal dose 50 %
LOAEC	Lowest observed adverse effect concentration
LOAEL	Lowest observed adverse effect level
Ni	Nickel
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level

NOEL	No Observed Effect Level
OECD	Organisation for Economic Co-operation and Development
OR	Odds ratio
PBPK/PBTK	Physiologically based pharmacokinetic (toxicokinetic) modelling
PM	Particulate matter
PND	Postnatal day
POD	Point of departure
RAC	Risk Assessment Committee
REACH	Registration, Evaluation and Authorisation of Chemicals
s.c.	Subcutaneous
SCD	Systematic contact dermatitis
SD	Standard Deviation
SIR	Standardized incidence ratio
SMR	Standardized mortality ratio
TDI	Tolerable daily intake
TRV	Toxicity (Toxicological) Reference Value – health-based, external exposure guidance value proposed by a European or relevant non-European institution (e.g. TDI, OEL).
TWA	Time weighted average
WHO	World Health Organisation

1 Introduction

Under PARC, WP4, project 4.1.3.1.a, human biomonitoring guidance values (HBM-GVs) are derived for priority substances. These HBM-GVs can be directly compared with HBM data and as jointly agreed values under PARC, they are a valuable tool for the harmonized assessment of health risks. The HBM-GVs are derived as needed for the general population and/or occupationally exposed adults.

The human biomonitoring guidance value for the general population (HBM-GV_{GenPop}) and the specific substance represents the concentration of the substance (or metabolite) in human biological material (mostly urine, rarely blood or other tissues) up to which, according to current knowledge - when considering individual substances - there is no risk of adverse health effects. An exceedance of this value should be a reason to investigate the causes and to minimize possible sources of exposure. The HBM-GVs derived for occupationally exposed adults (HBM-GV_{Worker}) represent a concentration of a substance or its relevant metabolite(s) in human biological media aiming to protect workers exposed to the respective substance regularly (each workday), and over the course of a working life from the adverse effects related to medium and long-term exposure (Apel et al., 2020).

In this document, an orientation HBM-GV-range for the general population is proposed and an HBM-GV for workers is derived for nickel and its compounds. Toxicology data is based on previous evaluations and studies published after the reports are included (ECHA, 2018; EFSA, 2015; EFSA, 2020; SCOEL, 2011; WHO, 2000, 2021). For the preparation of this document, reports and evaluations prepared by recognized organizations have been accessed. Critical studies for the evaluation were, if available, checked against the original reference. However, most of the toxicokinetic and toxicity studies were performed by companies; these studies are not published but are described in sufficient detail in the assessment reports and evaluated by competent authorities within the scope of the approval processes.

2 General information and physico-chemical properties of nickel

Nickel is a silvery-white, hard, ductile, metal that is magnetic at room temperature. It is generally found in its divalent form Ni^{2+} in minerals or with combination of other metals (ECHA, 2018). Ni^{2+} is the most relevant form of oxidation in the environment although it can be oxidated also into -1, 0, +1, +3, and +4 forms.

Both inorganic and organic nickel substances are mainly oxidized into +2, but in the cell, through redox reactions trivalent Ni^{3+} form of nickel might be produced. The oxidation state 0 is found in nickel tetracarbonyl.

The most commonly used nickel compounds in the EU are nickel, slags of ferronickel-manufacturing, nickel matte, nickel monoxide, nickel sulfate, nickel dichloride, nickel sulfide, nickel subsulfide, [carbonato(2-)]tetrahydroxy-trinickel, reaction mass of cobalt sulfide and nickel sulfide and nickel subsulfide, and nickel dinitrate (ECHA, 2018). Table IV.1, identifies and describes the physical properties of selected chemicals.

Table IV.4: Identification and chemical and physical properties (ECHA, 2018; PubChem, 2023)

Substance name	Nickel	Nickel dichloride	Nickel sulfate	Nickel dinitrate	Nickel oxide	Nickel subsulfide	Nickel tetra-carbonyl
Molecular formula	Ni	$NiCl_2$	$NiSO_4$	$Ni(NO_3)_2$	NiO	Ni_3S_2	$Ni(CO)_4$
CAS no	7440-02-0	7718-54-9	786-81-4	13138-45-9	1313-99-1	12035-72-2	13463-39-3
EC/list number	231-111-4	231-743-0	232-104-9	236-068-5	215-215-7	234-829-6	236-669-2
Appearance	Silvery-white, solid	Green/ brown/ yellow, solid crystalline	Yellow-green, solid crystalline	solid crystalline	Green-black solid crystals	Solid powder	Liquid
Molecular weight	58.693 g/mol	129.60 g/mol	154.76 g/mol	182.70 g/mol	74.693 g/mol	240.22 g/mol	170.73 g/mol
Melting point	1455 °C	1001 °C	840 °C decomposes	56.7 (hexaqua)	1984 °C	> 360 °C	-25 °C
Boiling point	2739 °C	973 °C sublimes	Decomposes	136.7 °C (hexaqua)	N/A	N/A	43 °C
Vapor pressure	0 mmHg	1 mm Hg at 671 °C (solid)	N/A	$3.4 \cdot 10^{-3}$ Pa, 5.0 °C	0 mm Hg at 20 °C	N/A	$17.07 \cdot 10^{3}$ Pa, 0 °C $77.68 \cdot 10^{3}$ Pa, 35.1 °C
Density at 20 °C	8.9 g/cm³	3.55 g/cm³	3.68 (anhyd) 2.07 (hexaqua) 1.95 (heptaqua)	2.05 (hexaqua) g/cm³	6.75 g/cm³	5.98 g/cm³	1.32 g/cm³
Solubility in water at 20 °C	Insoluble	Soluble 642 g/L	Soluble 293 g/L	Soluble 2385.0 g/L	Practically insoluble Ph: 6.9 , $27.1 \cdot 10^{-6}$ g/L	Practically insoluble Ph: 7.2 , $11.6 \cdot 10^{-3}$ g/L	Sparingly soluble 0.018 g/L

3 Use of nickel

Nickel is widely used to manufacture alloys such as stainless-steels alloys, nickel-copper alloys, nickel-chromium alloys, molybdenum-containing nickel alloys, and nickel-iron-chromium alloys (ECHA, 2018). Alloys can be used for different purposes such as electrical and electronics industries, coinage, and marine engineering. Other uses for nickel include:

- hard-wearing coatings are done by plating or electroforming. These coatings can be used for decorative or engineering purposes;
- manufacturing batteries such as nickel-cadmium, nickel/metal hydride alkaline batteries and lithium-ion batteries;
- catalysts;
- intermediates, mainly to manufacture catalysts;
- pigments;
- slags produced as a side-product of manufacturing ferronickel;
- welding. Nickel and its compounds can be welded and used in welding consumables.

4 EU legislation and regulation, classification

4.1 Legislation and regulation

The European Parliament and the Council have set an occupational limit value for nickel that is applied from the 18th of January, 2025 (Directive, 2022/431). The occupational exposure level has been set at 0.01 mg/m^3 for the respirable fraction and 0.05 mg/m^3 for the inhalable fraction, both measured as nickel. The use of nickel and its compounds in jewelry and articles in contact with skin has been restricted under Annex XVII of the REACH regulation.

The general requirements for materials and articles intended to come into contact with food are set in the Framework Regulation EC 1935/20044. In addition, it establishes good manufacturing practices for these materials and articles. Commission Regulation (EU) No 10/2011, food or food simulant from plastic materials and articles, limits migration of nickel to be 0.02 mg/kg. In addition, specific release limit of nickel from food contact materials and articles (0.14 mg/kg food) was set in the practical guide on metals and alloys used in food contact materials and articles, published by the Council of Europe in 2013.

The quality of water intended for human consumption is regulated by EU Council Directive 98/83/EC (Annex I, Part B 'Chemical parameters'), which gives a limit value of 20 µg/L for nickel. The same directive also indicates the minimum performance characteristics to be warranted by the method used for the analysis (Annex III). Commission Directive 2003/40/EC regulates the maximum limit for nickel in natural mineral water to be 20 µg/L. In addition, the performance characteristics to be warranted by the method used for the analysis are set in the Annex II.

4.2 Classification

Nickel and its compounds are classified as suspected human carcinogens (Carc. 2) or known human carcinogens (Carc. 1A), and skin sensitizers (ECHA, 2018). Many of these substances are also classified as respiratory sensitizers, reproductive toxicants (1B or 1A) and mutagens (Mut 2). Detailed classifications of selected substances can be found in table IV.2.

Table IV.5: Classification of nickel and its compounds

Substance name	Nickel	Nickel dichloride	Nickel sulfate	Nickel dinitrate	Nickel oxide	Nickel sub-sulfide	Nickel tetra-carbonyl
Hazard class and category	Carc. 2 STOT RE 1 Skin Sens. 1	Carc. 1A Muta. 2 Repr. 1B Acute Tox. 3 * Acute Tox. 3 * STOT RE 1 Skin Irrit. 2 Resp. Sens. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	Carc. 1A Muta. 2 Repr. 1B Acute Tox. 4 * Acute Tox. 4 * STOT RE 1 Skin Irrit. 2 Resp. Sens. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	Ox. Sol. 2 Carc. 1A Muta. 2 Repr. 1B Acute Tox. 4 * Acute Tox. 4 * STOT RE 1 Skin Irrit. 2 Eye Dam. 1 Resp. Sens. 1 Skin Sens. 1	Carc. 1A STOT RE 1 Skin Sens. 1 Aquatic Chronic 4	Carc. 1A Muta. 2 STOT RE 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	Flam. Liq. 2 Carc. 2 Repr. 1B Acute Tox. 2 * Aquatic Acute 1 Aquatic Chronic 1
Hazard statement code	H351 H372** H317	H350i H341 H360D *** H331 H301 H372 ** H315 H334 H317 H400 H410	H350i H341 H360D *** H332 H302 H372 ** H315 H334 H317 H400 H410	H350i H341 H360D *** H332 H372 ** H315 H318 H334 H317	H350i H372 ** H317 H413	H350i H341 H372 ** H317 H400 H410	H225 H351 H360D *** H330 H400 H410

5 Exposure

Nickel occurs naturally and anthropogenically in the environment (ECHA, 2018; EFSA, 2020). It is usually found in the form of nickel (II) or as a complex. The earth´s crust includes 0.08% nickel, which is mined for industrial use. Exposure to nickel might occur through air, food, drinking water, or occupational sources. Industry causes 80% of atmospheric nickel exposure. Thus, industrial sites have higher air nickel concentrations than non-industrial sites. At industrial sites, nickel air concentration ranges from tens to hundreds of ng/m^3, while at non-industrial sites, air concentration is ≤3 ng/m^3. Generally, nickel concentration in surface water has been ≤3 µg/L and in groundwater and drinking water it ranges from one to few tens µg/L. Nickel in soils can usually be detected at levels below 50 µg/kg, although in some regions, significantly higher concentrations are reached. Root vegetables may accumulate nickel in these contaminated areas that have high background soil concentrations.

EFSA (2020) has estimated nickel exposure through food and drinking water. The highest concentrations of nickel have been found in legumes, nuts, and oilseeds, but grains and grain-based products are the main sources for chronic exposure to nickel. Small children are chronically exposed to higher concentrations of nickel (maximum mean 12.5-14.5 µg/kg bw per day) than the elderly (maximum mean 3.77-4.29 µg/kg bw per day). However, the 95th percentile in infants might be up to 28.1-29.9 µg/kg bw per day. The main source for acute exposure has been beans, coffee, ready-to-eat soups, chocolate, and breakfast cereals. Acute exposure concentrations also vary significantly between the age groups, from the elderly at 1.89 µg/kg bw per day (95th percentile 5.35 µg/kg bw per day) to small children at 14.6 µg/kg bw per day (95th percentile 40.8 µg/kg bw per day). Nickel in drinking water had a mean upper bounder level of 3 µg/L, and the 95th percentile for upper bound level was 7 µg/L. From 500 ml of tap water, nickel exposure has been estimated to be 0.04 µg/kg bw per day and from 500 ml of bottled water, it´s 0.08 µg/kg bw per day. The TDI is 13 µg/kg bw.

The general population is mainly exposed orally, and inhalation is a minor exposure route (EFSA, 2020). The use of jewelry may result in dermal exposure. The main exposure routes for those occupationally exposed are inhalation and dermal (ECHA, 2018). In table IV.2, urine nickel concentrations in the general population are presented.

Table IV.6: General population urine nickel concentrations

Country (population)	Year when samples collected	n	Mean (µg Ni/L) *geom. mean	Range, P90/P95 (µg Ni/L)	Reference
Canada	2009-2011	5924	N/A	1.1 P95	(Saravanabhavan et al., 2017)
German	2020	102	1.35	4.0 P95	(Heitland and Köster, 2021)
German	2018	65	1.4	3.1 P90	(Schmied et al., 2021)
Germany-adult females	2005-2006	164	1.2	0.2-10.1 3.9 P95	(Darsow et al., 2012)
Germany-adult females, in polluted industrial area	2005-2006	579	2.1	8.1 P95	(Kasper-Sonnenberg et al., 2011)
France	2014-2016	2419 adults, 1052 children	1.53* 2.28*	5.10 P95 6.61 P95	(Oleko et al., 2024)
France	2008-2010	1992	2.00*	5.99 P95	(Nisse et al., 2017)

Country (population)	Year when samples collected	n	Mean (µg Ni/L) *geom. mean	Range, P90/P95 (µg Ni/L)	Reference
France	2006-2007	1991	1.23*	3.77 P95	(Fréry et al., 2010)
Belgium	2010-2011	1022	1.73*	4.73 P95	(Hoet et al., 2013)
Italy-Arezzo industrial	2011-2013	153	3.85*	0.53-15.33	(Chellini et al., 2017)
Italy-Arezzo-Urban	2011-2013	92	4.75*	0.54-18.32	(Chellini et al., 2017)
Italy-Arezzo-rural	2011-2013	55	2.71*	0.48-9.74	(Chellini et al., 2017)
Serbia-smokers	2000-2003	69	1.20	<0.01-8.20	(Stojanović et al., 2004)
Serbia-non-smokers	2000-2003	78	0.50	<0.01-4.60	(Stojanović et al., 2004)
Great Britain	published 2014	132	1.99 median	6.35 P95	(Morton et al., 2014)
Italy	2012-2015	260	1.47*	4.44 P95	(Aprea et al., 2018)
Finland	2020	147	1.5*	4.4 P95	(Porras et al., 2025)
HBM4EU**	2019-2020	104	1.4*	4.3 P95	Toda et al. unpublished
CDC (NHANES)	2017-2018	2791	1.1*	4.23 P95	(CDC, 2024)

**Control data from HBM4EU occupational studies

Some occupational exposure studies have been conducted to study inhalation and dermal exposure. The mean time-weighted average (TWA) for nickel inhalation exposure was 0.481 mg/m^3 in a Japanese battery plant (Yokota et al., 2007). Also, urine samples were measured pre- and post-shift, with results being 17.5 µg/L and 21.5 µg/L on the first day, and 20.1 µg/L and 20.9 µg/L on the second day of sampling, respectively. No correlation was detected between inhalation and urine nickel concentrations. In a retrospective study, nickel inhalation exposure was estimated to be 0.67 mg/m^3 in nickel alloy industry in the USA (Sivulka and Seilkop, 2009).

In Germany, the mean nickel inhalation exposure was 0.009 mg/m^3 (95th percentile 0.460 mg/m^3) across various industries (Kendzia et al., 2017). The highest concentrations were detected in gas metal and shielded metal arc welders (GM≥0.020mg/m^3). High alloyed welding fumes resulted in nickel air levels of 0.029 mg/m^3 in static samples at 6 hours exposure duration (Bertram et al., 2015). In a European study, nickel inhalation concentrations varied between work tasks, ranging from 0.5-3.9 mg/m^3 for inhalable dust and 0.02-0.77 for total nickel (Hughson et al., 2010). Packing nickel compounds had the lowest concentrations and stainless-steel production had the highest.

In the same study, dermal levels on hands and forearms ranged from 0.02-0.29 µg/cm^2 (GM) for soluble nickel and 0.08-8.40 for total nickel (Hughson et al., 2010). The highest concentrations were detected in packing nickel powder and the lowest in stainless steel production. In the study by Day et al. (2009) , dermal exposure on hands was greatest in powder-handling workers (24 µg), then in metal separation workers (20µg), and lowest in forming/machining workers (7 µg) during the production of cemented tungsten carbides.

Table IV.4: External and internal exposure of sparingly soluble nickel compounds. Modified from (ECHA, 2018). None of the original articles was available. MAG: metal active gas welding, MMA: manual metal arc welding, TIG: tungsten inert gas welding, * 68 %-range

Occupation	n (m=male, f=female)	Air concentration (µg/m³) mean (range)[SD]	Urine (µg/L)	Blood (µg/L)	Reference
Polishers of aeroplane parts	7 (6 m, 1 f)	26 (0.05-129.0) [41]	4.1 (0.5-9.5)		(Bernacki et al., 1978)
Grinders (nickel alloys)	8 (7m, 2f)	1.6 (0.02 –9.5) [3.0]	5.4 (2.1 – 8.8)		
Welders (MMA)	10 (7m, 3f)	6.0 (0.2 –46.0) [14.3]	6.3 (1.6 -14.0)		
Aviation mechanics	8 (4m, 4f)	52.0 (0.01 –252.0) [94]	12.2 (1.4 - 41.0)		
Flame sprayers	5 (4m, 1f)	2.4 (0.04- 6.5) [2.6]	17.2 (1.4-26.0)		
Nickel refining, roasting, melting	24	860.0 [1200]	65 [58]	7.2 [2.8]	(Høgetveit et al., 1980)
Nickel refining other areas	13	420.0 [490]	45 [27]	6.4 [1.9]	
Welders (high alloyed stainless steel; up to 34 % Ni) (MMA)	10 (m)	(30.0-1780.0) [150]	(7.8 - 26.5) [11.5]	(0.58-2.9) [1.2]	(Rahkonen et al., 1983)
Nickel catalyst production	73	(<200.0–5870.0)	(9.0-300.0) [64]		(Zwennis and Franssen, 1983)
Stainless steel welders (TIG)	10 (m)	median 2.3 (0.7-0.9)	median 4-6 (1.4-9.5)		(Zober et al., 1984)
Smelting ores containing nickel					(Morgan and Rouge, 1984)
Drying of nickel oxides	67	(10.0-5000) [310]	24 [24]		
Drying of nickel oxides	37			8.9 [5.9]	
Production of nickel powder	48	(90.0-1530) [310]			
Production of nickel powder	25			7.2 [4.8]	
Contact with sparingly soluble nickel compounds	15 7	(220-4180) [1540]	39 [28]	7.4 [5.1]	
Welding of high alloyed steel (75 % Ni)	11 (m)	440 (70.0-1100)	18 (9.7-38.0)		(Akesson and Skerfving, 1985)

Occupation	n (m=male, f=female)	Air concentration (µg/m³) mean (range)[SD]	Urine (µg/L)	Blood (µg/L)	Reference
Welders (stainless steel) (MMA)	41 (m)	(95% <500)	median 23.8 (2.5-144.0)		(Zober and Weltle, 1985)
Hollow glass industry-mould manufacturing +maintenance					(Zober and Weltle, 1985)
Mechanics	406	(18- 3800)	(2.9-24.3) [7.4]		
Mechanics	104	(18- 3800)		0.9 (0.75–2.05)	
Powder flame sprayers	114	(3 -600)	(8.5-81.5) [25.3]		
Powder flame sprayers	40	(3 -600)		(0.75-3.25) [1.95]	
Combined jobs	394	(300-410)	(0.75-3.25) [17.5]		
Combined jobs	108	(300-410)		(0.75-4.10) [1.65]	
Stainless steel welders (MAG, MMA)	103 (m)	93 (<50- 320) [81.4] median 60	18.5 (0.1-209.4) [28.5] median 10.2	4.9 (<1.8-19.6) [4.0] median 3.9	(Angerer and Lehnert, 1990)
Stainless-steel welders					(Emmerling et al., 1989)
MMA	61	median 24 (7.4-52.7)*	median 10.1 (3.4-20.1)*	(0.4-3.2)*	
MAG	46	median 68.6 (22.1-238.7)*	14.0 (4.4-34.3)*	(0.7-2.8)*	
TIG	16	8 (2.1-20.3)*	5.7 (1.8-15.4)*	(0.2-2.7)*	

m=males, f=females, MMA=Manual metal arc welding, MAG=Metal active gas welding, TIG=Tungsten inert gas welding

Table IV.7: External and internal exposure to soluble nickel compounds. Modified from (ECHA, 2018)

Occupation	n (m=male, f=female)	Air concentration (µg/m³) mean (range)[SD]	Urine (µg/L)	Blood (µg/L)	Reference
Electroplating	21(m)		27.5 ± 21.2 (3.6–65)		(Bernacki et al., 1978)
Electroplating	90 (m)	0.23 ± 0.42	129.2 ± 105.6	11.9 ± 8.0	(Høgetveit et al., 1978)
Electroplating (NiSO4)	4 (m), 1 (f)	(30–160)	6.6 (3–10)	1.2 (< DL-1.7)	(Tola et al., 1979)
Electroplating	66 (m) (personal air sampling), 68 (m) (measurement in the urine)	0.5 ± 2.47 (0.01–20)	65 ± 42 (10 200)	N/A	(Morgan and Rouge, 1979)
Electroplating	5 (m) (personal air sampling), 8 (f) (measurement in the urine)	9.3 ± 4.4 (4.7–16.4) (measured over 3 days)	34 ± 32 (7–142) (before shift) 64 ± 63 (9–262) (during shift) 46 ± 32 (5–139) (after shift)	N/A	(Bernacki et al., 1980)
Electroplating	3 (m)	(0.02–0.14) (during a working day)	(25–255) (during a working day)	(2.5–18.5) (during a working day)	(Høgetveit et al., 1980)
Electroplating	15(m), 4 (f)	N/A	27.1 ± 21.2 (3.1–82)	N/A	(Sunderman et al., 1986)
Battery factory (Ni-H2)	4 (m), 3 (f)	N/A	32.2 ± 40.4 (2.8–103)	N/A	
Electroplating ($NiSO_4$, $NiCl_2$)	11 (m)	N/A	28 ± 4	3.8 ± 0.5	(Sunderman et al., 1988)
Electroplating ($NiSO_4$)	10 (m)	(2.8–116.7)	10.5 ± 7.5 (before shift) 20.6 ± 18.1 (after shift)	N/A	(Oliveira et al., 2000)

m=males, f=females, N/A=not available

6 Toxicological profile of Nickel

6.1 Toxicokinetics

6.1.1 Absorption

Inhalation exposure to nickel compounds is in the form of aerosols (dust) or attached particles (ECHA, 2018). Nickel particles that are <100µm may enter the respiratory tract. Larger particles (5-30 µm) stay in the upper airways, but smaller particles go into the bronchiolar region and only particles <1 µm enter the alveolar region. In humans, 20-35% of inhaled nickel is absorbed, and the rest of the nickel is either swallowed, expectorated, or stays in the lungs. The solubility of nickel affects absorption. Greater concentrations of nickel have been detected in the urine after exposure to soluble nickel compounds than after exposure to insoluble nickel compounds. Thus, soluble nickel compounds are more readily absorbed from the lungs than insoluble nickel compounds.

For the general population, oral exposure is the main route of exposure (ATSDR, 2005; ECHA, 2018; EFSA, 2020). However, due to mucociliary clearance, it is also an important route of occupational exposure (ATSDR, 2005; ECHA, 2018; EFSA, 2020). The gastrointestinal absorption of nickel is affected by the solubility of nickel, fasting status, and food. Absorption is higher for soluble nickel compounds and during the fasting state. Absorption decreases for less-soluble nickel compounds and if nickel has been consumed at the same time with food; especially milk, coffee, tea, and orange juice decrease the solubility. However, soft drinks might increase the solubility. The peak level of nickel in blood was reached one hour after oral administration of nickel-supplemented water in the fasting state (Nielsen et al., 1999). When nickel-supplemented water was administered with food, peak level of nickel in blood was reached in three hours and the peak was 13-fold lower than when administered without food (Nielsen et al., 1999). Overall, oral absorption is affected by several aspects (nickel form, feeding state, and the species), thus, bioavailability also varies between 1-40% (ECHA, 2018). Unabsorbed nickel is excreted into feces.

In animals, 1-10% of nickel, nickel chloride, or nickel sulfate is absorbed from the gastrointestinal tract (ECHA, 2018). The peak urine concentration of nickel chloride in Fischer-344 rats was observed four hours after administration, and the dose was eliminated in 48 hours (TERA, 1999).

In animals, soluble nickel compounds are absorbed into the cell through the metal ion transport system or membrane diffusion, while insoluble nickel compounds are phagocytized (ECHA, 2018).

Nickel has only limited ability to penetrate through the skin, and solubility, occlusion, skin damage, or nanoparticle form of the nickel may increase penetration in humans (ATSDR, 2005; Crosera et al., 2016; ECHA, 2018). Large parts of nickel remain either on the skin surface or in the stratum corneum (ECHA, 2018). Animal studies support the human observations that nickel may penetrate through skin (ECHA, 2018). In animals, skin absorption has been higher than in the humans.

6.1.2 Distribution and metabolism

After absorption, nickel is bound to albumin and metalloproteins and is mainly distributed into the lungs, thyroid, and adrenal glands but also into brain, kidneys, heart, liver, spleen, and pancreas (ATSDR, 2005; EFSA, 2020). Workers exposed through inhalation have a higher amount of nickel in their lungs compared to the general population. The lung burden is even higher for less-soluble compounds than for soluble compounds. In addition, less-soluble compounds tend to remain in the nasal mucosa. Studies in rodents, show that nickel cross the placenta (ATSDR, 2005). In addition, fetal tissues have had similar levels of nickel as in adults (WHO, 2021). Nickel may also cross blood-brain-barrier via the divalent metal transporter 1 (DMT1) (EFSA, 2020). In animals, soluble nickel compounds are mainly found in the kidneys but also in the liver, heart, lung, fat, peripheral nerve tissues, and in the brain regardless of the exposure route (ECHA, 2018).

Occupational exposure increases serum nickel concentration when compared to the general population (ATSDR, 2005). In addition, exposure to soluble nickel compounds leads to higher serum nickel

concentrations compared to less-soluble compounds in workers. Sensitivity towards nickel does not seem to affect nickel concentrations in hair, urine, or serum.

Nickel metal is not metabolized, although extracellular metabolism after binding to plasma albumin does exist (ATSDR, 2005; WHO, 2021). Nickel binds to the albumin´s histidine residue at the third position from the amino terminus, and this binding is similar in humans, rats, and bovines, but dogs do not have it. It has been modelled that nickel is removed from albumin to histidine via a ternary complex composed of albumin, nickel, and L-histidine. The composed L-histidine nickel complex can pass through biological membranes. However, nickel that is tightly bound to α-macroglobulin is nonexchangeable.

6.1.3 Excretion

Absorbed nickel is excreted in urine regardless of the exposure route (ATSDR, 2005). In addition, some of the nickel might be excreted into breast milk. Nickel that is not absorbed is excreted in feces.

The clearance of lungs has been studied in animals, and it highly depends on the dosed form of nickel (ATSDR, 2005). After intratracheal administration, it was shown that the less-soluble nickel compound, nickel subsulfide, had two half-times: 38% had a half-time of 1.2 days, 42% had a half-time of 12.4 days, and 10% remained after 35 days. Soluble nickel chloride was mainly cleared within the first 24 hours (71%). The half-life in the lungs for nickel oxide has been over 100 days (ECHA, 2018).

Nickel oxide is excreted only into feces, while nickel subsulfide is excreted into both urine and feces (ECHA, 2018). Thus, nickel oxide is removed from the lungs through macrophage mediated process.

After oral exposure, 27% of the nickel dose has been excreted in the urine within three days when administered only with water. However, if nickel was administered with food only 2% was excreted into the urine within three days (EFSA, 2020). Food markedly reduces the absorption of nickel from the gastrointestinal tract. The elimination half-life has been estimated to be 28 ± 9 hours in human volunteers (EFSA, 2020).

6.1.4 PBPK modelling

Sunderman et al. (1989) developed a PBPK model for oral exposure. The model was modified from a multicompartment animal model. The developed model was based on two human experiments involving eight human volunteers. The volunteers' serum, urine, and fecal nickel concentrations were determined two days before and four days after the administration of nickel sulfate (12,18 or 50 µg Ni/kg bw in water or in food to the same subjects). The model was four-compartment (gut, serum, tissues, and urine) model. The model predicted the dataset well, but it was not tested with an independent dataset.

Dede et al. (2018) updated the model from Sunderman et al. (1989) and added a feces compartment to the model. Dede et al. (2018) model was tested with data from Sunderman et al. (1989) and Nielsen et al. (1999). The model fit well with the Sunderman et al. (1989) data but showed some variation with the Nielsen et al. (1999) data. The Nielsen et al. (1999) model had a higher absorption rate than the Sunderman et al. (1989) model (2.95% vs. 0.7%). The most sensitive parameter for the Dede et al. PBPK model was related to the oral absorption of nickel, and another sensitive parameter was the urine elimination rate.

A model for deposition and clearance of nickel from the human lung was developed by Hsieh et al. (1999b). This one-compartment model can calculate lung burden for nickel in the different occupational ventilation conditions and exposure concentrations. Yu et al. (2001) modified the model by adding inhalability, a mixed breathing mode, and a clearance rate coefficient, and then validated the human model. The Hsieh et al. (1999b) human model was based on a one-compartment dosimetric model of nickel deposition and clearance in rat lungs, which had a good fit with the predicted and measured lung burdens (Hsieh et al., 1999a). Acute toxicity

A small child (2-year-old) accidentally ingested nickel sulfate (570mg/kg), which caused cardiac arrest after 4 hours and death after 8 hours of exposure (ATSDR, 2005). A man died from Adult Respiratory Distress Syndrome (ARDS), 13 days after spraying nickel using a thermal arc process (Rendall et al., 1994). Exposure

to nickel was estimated to be 382.1 mg/m^3 and the nickel was mainly in fine particle form. During 90 minutes of exposure, the overall inhaled dose was estimated to be 1 g. Drinking water contaminated with nickel sulfate and nickel chloride, with total nickel amount 1.63 g/L, caused an intoxication of 32 electroplating workers (Sunderman et al., 1988). Symptoms include nausea, vomiting, abdominal discomfort, diarrhea, giddiness, lassitude, headache, cough, and shortness of breath. Symptoms occurred from a few hours up to 2 days and exposure ranged 0.5-2.5 g of nickel. Blood nickel concentration was 13-1340 µg/L and urine nickel concentration ranged from 0.15-12 mg/g creatinine. Dialysis patients were exposed to nickel during hemodialysis due to the leaching of a nickel-plated stainless steel water heater tank (Webster et al., 1980). Nickel plasma concentrations were 3 mg/L and symptoms included nausea, vomiting, weakness, headache, and palpitations.

Acute toxicity studies in animals show that soluble nickel compounds are more toxic than poorly soluble nickel compounds (ECHA, 2018). Oral LD50 values for nickel sulfate have been 39-190 mg Ni/kg bw, for nickel chloride 43-130 mg Ni/kg bw, nickel nitrate >303 mg Ni/kg bw, nickel acetate 116-325 mg Ni /kg bw, nickel carbonate 402-465 mg Ni/kg bw, metallic nickel >1472 mg Ni/kg bw, and for nickel hydroxide >1000 mg/kg bw. The oral LD50 for nickel oxide, nickel dihydroxide, nickel trioxide, nickelsulfide, and nickel subsulfide is >2000 mg Ni/kg bw, and for nickel oxide black or green, it is 8796 - >11 000 mg Ni/kg bw. Nickel oxide nanoparticles had an LD50 >5000 mg/kg bw studied in rats (Lyons-Darden et al., 2023). Metallic nickel nanoparticles (diameter 50 nm) caused the death of female and male mice (8/8) at dose level of 2000 mg/kg bw, and with an average diameter of 70 nm, 5/8 female and male mice died at the same dose level (Shipelin et al., 2022).

ECHA (2018) has listed animal inhalation acute toxicity studies. There are only few inhalation acute toxicity studies and more subacute toxicity studies. The use of subacute toxicity studies provides more conservative estimate than acute toxicity studies. Metallic nickel has an LC50 >24 mg Ni/m^3 in the subacute toxicity study (ECHA, 2018). Nickel sulfate has caused the death of rats at dose level ranging from 3.3-12.2, and in mice at dose levels ranging from 1.4-1.7 mg/m^3 in 12–16-day studies (ECHA, 2018; NTP, 1996c). No NOAEC could be determined either in rats or mice (ECHA, 2018; NTP, 1996c). Nickel subsulfate has caused deaths at dose of 0.97 mg Ni/m^3 after 6 hours of exposure (ECHA, 2018). Nickel subsulfide caused deaths in rats at 7.33 mg Ni/m^3, no NOAEC was identified. In mice, deaths were seen at 7.33 mg Ni/m^3, and the NOAEC was 0.44 mg Ni/m^3 in 16-day study (NTP, 1996b). Nickel oxide did not cause deaths at dose levels >23.6 mg Ni/m^3 in rats or mice in 16-day studies (NTP, 1996a). No NOAEC was identified for rats and NOAEC for mice was 0.9 mg/m^3 (NTP, 1996a). For nickel oxide nanoparticles the LC50 was found to be >5.42 mg/L equivalent to 5420 mg/m^3 (Lyons-Darden et al., 2023). In other studies, nickel oxide nanoparticles have caused inflammatory responses and changes in lung flora after acute exposure to doses of 20 µg/mouse, 4mg/kg in mice, and 50 cm^2/rat given by pharyngeal aspiration, oropharyngeal aspiration, or intratracheally instilled, respectively (ECHA, 2018; Glista-Baker et al., 2014; Jeong et al., 2022; Sager et al., 2016). Nickel dust (no specification of nickel compounds given) resulted in an LC50 value of 15 mg/m^3 (time not given) in guinea pigs. A two hour exposure to nickel chloride at 0.25-0.20 mg/m^3 has resulted in the suppression of immune responses (ECHA, 2018).

No dermal acute toxicity studies are available.

In summary, acute toxicity varies among nickel compounds based on their solubility. Soluble nickel compounds are acutely toxic, cat 2 (i.e. nickel sulfate, nickel chloride), and less soluble compounds (metallic nickel, nickel oxide) are not acutely toxic (cat. 4) when orally exposed. Although the acute inhalation toxicity of poorly soluble metallic nickel and nickel oxide can be estimated to be low, the data is limited. No data is available for dermal acute toxicity.

6.2 Irritancy and corrosivity

Irritation and corrosivity have been summarized previously by ECHA (2018) and the Danish EPA (2013). The main points of these reports are summarized below. Nickel octanoate and nickel diperchlorate have been classified as corrosives. Metallic nickel is not a skin or eye irritant, but metallic nickel powder may mechanically cause irritation. Thus, it is suggested to be an eye irritant. There are some inconsistencies in the data on the irritancy of soluble nickel salts, but overall data suggest that soluble nickel salts are skin irritants. In addition, nickel sulfate has been studied to be a slight eye irritant, but nickel nitrate hexahydrate is a severe eye irritant (Nielsen and Larsen, 2013). Nickel may also cause respiratory tract irritation, including allergic reactions (such as asthma), and mucosal irritation (ECHA, 2018). The skin and/or mucosal irritation may be clinically indistinguishable from allergies. Respiratory effects are discussed in the chronic toxicity chapter.

Kalima and Lammintausta (1984) studied the irritation caused by nickel chloride (2.5 and 5 %) and nickel sulfate (5 %) in a 24- and 48-hour patch test. A 24-hour test was only conducted for nickel sulfate. Both substances caused irritation, but nickel chloride was more irritant than nickel sulfate. Nickel chloride was already irritant at 2.5 % solution. In total, 11% of the tested individuals had an irritation reaction after nickel exposure. In another study, where the skin was scarficated, nickel sulfate was only marginally irritant at a dose level of 0.13 % but was very irritant at a dose level of 1 % (Frosch and Kligman, 1976).

6.3 Skin sensitization

The ability of nickel and its compounds to cause respiratory sensitization has been described in the chapter “Chronic toxicity”. This chapter focuses on skin sensitization and acute systemic contact dermatitis.

Soluble and insoluble nickel are skin sensitizers and cause allergic reactions (ECHA, 2018). These reactions have been documented in the general population as well as in the occupational population (ECHA, 2018). The most common type is allergic contact dermatitis (type IV hypersensitivity) in the general population. ECHA (2018) reports an increase in the incidence of skin reactions in the general population worldwide, but from the EU opposite reports are described during recent years. The incidence of skin reactions has mostly been between 11.8-14.3%. In table IV.6, human skin sensitization studies published since 2018 are listed. Most of these studies were done using data from patients who were studied for allergic dermatitis. Nickel sulfate concentrations of 2.5% (North America) or 5% (Europe) were used in the patch test but not all the studies specified the concentration. A few studies included both concentrations, and it was found that a more positive reaction is received if 5% of nickel sulfate is used in the patch test (Goldminz and Scheinman, 2018; Svedman et al., 2022). The range for nickel sulfate positivity in patch test was between 6.4% and 33%. Basketter (2021) reviewed guinea pig skin sensitization studies (Maximization test, Draize test, Freund's complete adjuvant, Optimization test, Open epicutaneous test, and TINA test). They concluded that positivity for nickel in these tests varied between 0-55%, leading to a weak/moderate sensitizer. Local lymph node assays (LLNA) were also reviewed, and the results were contradictory, with some indicating nickel as a non-skin sensitizer and others as a weak/moderate skin sensitizer. Overall, Basketter et al. stated nickel weak/moderate sensitizer based on in vivo studies. In addition, they concluded that animal studies are not the best predictors for nickel skin sensitization due to a human-specific mechanistic route for nickel sensitization that animals lack.

Nickel may cause acute systemic contact dermatitis (SCD) after oral exposure of previously nickel sensitized persons (EFSA, 2015; EFSA, 2020; WHO, 2021). In the SCD, eczematous flare-up reactions occur in the skin after oral exposure to nickel (WHO, 2021). SCD has occurred at the dose levels of 0.3-0.5 mg of nickel and dose-relationship at low dose exposures has been suggested (WHO, 2021). These effects have been seen in case-control studies or in volunteers’ studies but the sample size has been small in most of the studies. EFSA has performed a BMD modelling based on two studies: Gawkrodger et al. (1986) and Jensen et al. (2003) (EFSA, 2020). Analysis resulted in the BMDL10-BMDU10 interval of 0.0124-2.43 mg/nickel/person. Large BMDL-BMDU range indicates high uncertainty and is likely due to small size of the studies despite of multiple dose groups. In addition, BMDL10 0.0124 mg nickel/person was outside of the dose range of the studies.

Thus, EFSA concluded that no guidance value for acute effects can be derived based on the BMD modelling. EFSA identified LOAEL of 4.3 µg/kg bw based on the Jensen et al. study. However, no acute reference dose was set due to insufficient data. In the recent evaluation of ATSDR (2024), no minimal risk level for acute effects of oral exposure was derived due to insufficient data. They also evaluated the possibility to use SCD as a basis for acute minimal risk level of oral exposure.

In addition to skin and respiratory reactions, EFSA (2015, 2020) states that oral exposure to nickel may cause systemic nickel allergy syndrome (SNAS). In this syndrome, different kinds of adverse reactions may occur such as gastrointestinal (abdominal pain, diarrhea and/or constipation, nausea and/or vomiting), atypical systemic manifestations (headache, chronic fatigue), and chronic dermatological symptoms (urticaria-angioedema). However, relationship with oral exposure to nickel has not been confirmed and no dose-response is available.

Table IV.8: Skin sensitization studies

Study	Substance	Study design	Results	Reference
Retrospective cross-sectional	Nickel sulfate hexahydrate, 2.5 %	North America, 2001-2008, hand dermatitis (n=9966), food dermatitis (n=1276), hand and food dermatitis (n=1625), 48-hour patch test, result read on day 2, day 3-7	Hand dermatitis group: 10.3 % were positive in patch test. Food dermatitis group: 6.4 % were positive in patch test. Hand and food dermatitis group: 8.9 % were positive in patch test.	(Silverberg et al., 2022)
Retrospective cross-sectional	Nickel sulfate hexahydrate 2.5 %	North America, 2000-2016, hand eczema (n=9986), no hand eczema (n=26976), 48-hour patch test, result read on day 2, day 3, and day 7	Hand eczema group: 17.9 % were positive in patch test. No hand eczema group: 18 % were positive in patch test.	(Silverberg et al., 2021a)
Retrospective cross-sectional	Nickel sulfate hexahydrate 2.5 %	North America, 1994-2016, eyelid dermatitis (n=1137), no eyelid dermatitis (n=13840), 48-hour patch test, result read on day 2, day 3, and day 7	Eyelid dermatitis group: 18.56 % were positive in patch test. No eyelid dermatitis group: 17.30 % were positive in patch test.	(Warshaw et al., 2021)
Retrospective cross-sectional	Nickel sulfate hexahydrate 2.5 %	North America, 2000-2016, children, allergic contact dermatitis suspicion (n=1634), hand eczema patients (n=237), patch test	No hand eczema (n=1382): 26.9 % were positive in patch test. Hand eczema: 18.2 %	(Silverberg et al., 2021b)
Cross-sectional multi-center study	Nickel sulfate hexahydrate 5 %	Swedish, during 2018, adults, n=1356 (m=431, n=925), 48-hour patch test, results read on day 3 or 4, and day 7	13.3 % of all tested were positive for nickel. 17.2 % of females were positive and 4.9 % of males.	(Isaksson et al., 2021)

Study	Substance	Study design	Results	Reference
Cross-sectional	Nickel sulfate hexahydrate 5 %	Lithuania, metal plant workers, 75 production workers, 60 office staff, 48-hour patch test, result read on day 3 or 4 and day 7	11.1% of metal workers were positive and 9.33 % of office workers were positive.	(Linauskiene et al., 2020)
Cross-sectional, retrospective	Nickel sulfate 5 %	Greek, 2014-1016, n=1978 (m=619, n=1359), divided in blue collar and white-collar workers, 48-hour patch test, result read on day 2,3 or 4 and day 7	From blue-collar population 7.33 % in relation to total population was positive. From white collar population 18.55 % in relation to total population was positive.	(Tagka et al., 2021)
Cross-sectional	Nickel sulfate	Contact dermatitis patients, children aged 1-17, n=80, patch test	14.8 % of patients were positive.	(Yücel and Özçeker, 2021)
Cross-sectional, retrospective, multi-center	Nickel sulfate 5 %	Denmark, n=1573, 2012-2016, 48-hour patch test, result read on day 2,3 or 4 and day 7	7.0 % were positive in patch test. Result decreased significantly from the previous study performed in 2003-2011 when 9.7 % were positive in patch test for nickel.	(Simonsen et al., 2018)
Prospective	Nickel sulfate hexahydrate 2.5 % and 5 %	2 clinics in North America, dermatitis patients, n=2287, patch test	2.5 %: contact allergy in 16.8 % of patients 5 %: contact allergy in 20.3 % of patients	(Svedman et al., 2022)
Registry study	Nickel sulfate	North America, 2018-2019 (1-year), Contact allergen management program (CAMP) database, n=32 220, patch test	19.6 % were positive in patch test.	(Scheman et al., 2020)
Case-study	Nickel sulfate 5 % at patch test	Welding and grinding workers, n=9, males, patch test	Nickel dust (insoluble nickel) at workplace was greater than TWA 0.2 mg/m^3 in 16.7 % of the samples (max 0.34 mg/m^3). Erythematous papules/patches and itching in various areas of the body after work. 2/9 were positive for patch test and concluded to have allergic contact dermatitis, and rest to have irritant contact dermatitis.	(Kim et al., 2022)

Study	Substance	Study design	Results	Reference
Cross-sectional	Nickel sulfate	Spain, 1466 hand eczema patients, patch test	20 % of patients with atopic dermatitis were positive in patch test and 27 % without atopic dermatitis.	(Mora-Fernández et al., 2022)
Cross-sectional	Nickel (not specified)	India, 100 hand eczema patients, patch test	16 % of hand eczema patients were positive in patch test	(Kataria et al., 2020)
Cross-sectional	Nickel sulfate hexahydrate 5 %	Crete, 75 patients, 48-hour patch test, result read on day 2, and day 4	17.3 % were positive in patch test	(Koumaki et al., 2020)
Retrospective	Nickel sulfate	Sri Lanka, 2012-2018, n=438, 48-hour patch test, result read on day 2, and day 4	16 % were positive in patch test	(Keragala et al., 2019)
Prospective	Nickel sulfate 2.5 % and 5 %	North America, 2017-2018, n=205, patch test	2.5 %: 20 % were positive in patch test. 5 %: 31 % were positive in patch test.	(Goldminz and Scheinman, 2018)
Retrospective	Nickel sulfate	Latin America, 2013-2020, 173 hand eczema patients, patch test, result read on day 2, and day 4	33 % were positive in patch test.	(Suzuki et al., 2023)
Retrospective cohort	Nickel sulfate	Israel, 2016-2018, 517 patients, patch test, results read on day 2 and day 3	20,3 % were positive in patch test.	(Rosenberg-Bezalel et al., 2022)
Retrospective cross-sectional	Nickel sulfate 2.5 %	North America, 1998-2016, n= 43582, patch test	18.2 % of total individuals were positive in patch test and from this population 3.4 % had occupationally related nickel allergy. Most sensitized occupations were hairdressers/barbers/ cosmeticians (14.3 %), machine operators (9.3 %), health care occupations (7.1 %) and precision metal workers (6.7 %). Industries that persons were most sensitized to nickel were durable good manufacturing (24.6 %), personal services (15.7 %), retail trade (9.3 %) and health care services (8.2 %).	(Warshaw et al., 2019)

Study	Substance	Study design	Results	Reference
Retrospective	Nickel sulfate 5 %	Italy, 2008-2018, patients suspected to have allergic contact dermatitis n=11022, patch test, results read on day 2 and day 4 and in doubtful cases on day 7.	From total number of tested 15.0 % were positive in patch test. From atopic dermatitis patients (n=438), 29.2 % were positive in patch test and patients with eczema other than atopic dermatitis (n=4810) 31.6 % were positive in patch test.	(Napolitano et al., 2019)
Retrospective cross-sectional	Nickel sulfate 2.5 %	Mayo Clinic /USA, 2011-2015, patients with contact dermatitis n=2565, 48-h patch test, results read on day 2, and day 4-7.	18.3 % had allergic reaction in patch test. From this group, 52.2 % had weak reaction, 31.3 had strong reaction and 16.4 % had extreme reaction. 1.4 % had irritant reaction in patch test.	(Veverka et al., 2018)

6.4 Chronic toxicity

6.4.1 Human data

In humans, there is evidence that nickel is associated with pulmonary fibrosis (Berge and Skyberg, 2003) and asthma (ATSDR, 2005; ECHA, 2018). In addition, nickel is a lung carcinogen, but this topic is discussed in its own chapter; thus, this chapter includes only nonmalignant endpoints.

6.4.2 Human data, general population

In the general population, an association between nickel and asthma has been found, although contradictory findings also exist. A few studies also indicate an association between nickel and metabolic diseases, as well as impaired kidney function.

Rosa et al. (2016) studied the association between personal air levels of metals and respiratory health in adolescents aged 11-14 years (n=410) in the industrial province of Brescia, Italy. The study included three former and current industrial sites and a non-industrial site. The adjusted model showed an association between parental reported asthma and nickel air concentration (RR 1.11, 95 % CI [1.03, 1.21] per 4 ng/m^3 increase). The adjustment was done for maternal asthma, child's sex, child's age, SES status, and PM^{10} concentration. Joh et al. (2021) found a dose-response between blood nickel concentration and pulmonary function in Korean men aged ≥40 but not in women. In a German study (n=2051, age 19-24), self-reported nickel allergy was associated with the incidence of wheezing (adjusted OR 2.26, 95% CI 1.10-4.62) in both men and women (Kolberg et al., 2020). An association was also found between nickel allergy and asthma, but only in males (adjusted OR 4.34, 95% CI 1.22-15.41). However, no association between increased asthma-risk and nickel exposure was observed in a case-control study in China (Huang et al., 2016). After adjusting for potential confounders (such as age, gender, tobacco smoking, educational level, occupational dust exposure, family history of asthma, pet ownership, flower gardening, physical activity, and body mass index) the urine nickel concentration was found to be negatively associated with asthma OR 0.30 (0.22, 0.41).

In a recent, Danish nationwide cohort study, the association between long-term exposure to air nickel in $PM^{2.5}$ and mortality was studied (So et al., 2023). Nickel was associated with lung cancer mortality, but not with all natural causes of death, cardiovascular diseases, or respiratory diseases. In another similar study that included six European cohorts (Belgian, Danish, Dutch, Norwegian, Rome, and Swiss) from the ELAPSE study, an association was found between mortality from natural causes and nickel. However, this association did not remain after adjusting for $PM^{2.5}/NO_2$ (Rodopoulou et al., 2022).

In the NHANES study of 2017-2018, the association between nickel and diabetes was studied in the general population (n=1585) (Titcomb et al., 2021). The study population was divided into four quartiles based on their urine nickel concentration. The odds ratio for diabetes was 2.7 (p=0.03) when the highest quartile (U-Ni >1.94 µg/L) was compared to the lowest (U-Ni <0.65 µg/L) after adjusting for age, sex, race/ethnicity, and urine creatinine concentrations, in addition to demographic, socioeconomic, and lifestyle factors. In addition, urine nickel concentration was positively associated with a male's body mass index and waist circumference (Wang et al., 2023). In another study, using the same NHANES data cohort (n=1588), urine nickel concentration was linked with impaired kidney function (Nan et al., 2022).

6.4.3 Human data, occupationally exposed populations

It has been clearly shown that nickel causes occupational asthma (ECHA, 2018). Asthmatic response may be a rapid onset attack (antibody-mediated Type I hypersensitivity) associated with acute bronchospasm, a late response reaction at 6-12 hours after exposure (antigen-antibody immune complex-mediated inflammatory reaction), or combination of these two reactions. Case studies have linked nickel induced asthma to specific IgE mediated immune response (ECHA, 2018). However, only few studies show specific IgE, positive skin tests and positive provocation tests with nickel sulfate in exposed persons (ECHA, 2018). Hannu et al. (2007) reported 34 diagnosed occupational asthma cases due to exposure of stainless steel welding fumes at Finnish

Occupational Health Institute during 1994-2003. Occupational asthma incidence was estimated to be 0.2-2.0 per 1000 per year in Finland.

Syurin and Vinnikov (2022) studied nickel pyrometallurgical workers (n=1424) in an observational prospective study from 2007-2021 at a large nickel production plant in the Russian High North. The most common diseases were chronic bronchitis (3.81 cases/1000 workers/year), sensorineural deafness (2.36 cases/1000 workers/year), and musculoskeletal disorders (1.90 cases/1000 workers/year). However, these results were not adjusted for confounding factors, and smoking was found to be markedly associated with each of these diseases. They also found that an air nickel concentration equal to or above 0.95 mg/m^3 predicted any occupational disease claim when adjusted for smoking, baseline chronic bronchitis and baseline sensorineural deafness (HR 2.12, 95% CI 1.35–3.32), but adjustments for other metals were not made.

Muir et al. (1993) studied 745 workers at the Ontario Copper Cliff sinter plant who were exposed to airborne dust containing nickel up to 100 mg/m^3. They examined the effects on the lungs with x-rays according to the ILO classification. No information on the confounding factors was available. The prevalence of small irregular lung opacities was within the range identified in cigarette smokers or in workers exposed to dusts of low fibrogenicity. Another study, that included 1046 nickel refinery workers in Norway, found an increased risk for pulmonary fibrosis with cumulative exposure to soluble nickel or sulfidic nickel (Berge and Skyberg, 2003). When adjusted for time, age, and smoking (pack-years) cumulative exposure to soluble nickel predicted a 10% and sulfidic nickel 15% increase in the prevalence of an ILO score > 1/0 per 1 mg/m^3 –year. After correcting for the sampler cumulative exposure levels corresponded to average exposure levels of 0.17 and 0.6 mg/m^3 inhalable fraction for soluble and sulfidic Ni, respectively.

Most of the mortality studies did not find an association with nonmalignant respiratory diseases (ATSDR, 2005; ECHA, 2018). A study in Ontario involving non-sinter workers in nickel refineries found increased mortality due to pneumoconiosis, silicosis/anthracosilicosis after 15 years of working, but 80 % of these deaths were in underground miners presumably exposed to crystalline silica (Lightfoot et al., 2017). Sorahan and Williams (2005) did not find an increased risk for mortality from non-malignant respiratory diseases in nickel refinery workers. Similarly, Arena et al. (1998) did not find an increased risk for mortality from non-malignant respiratory diseases in metallic alloy production workers exposed to nickel. In addition, Moulin et al. (1990) did not show an increased risk for mortality from non-malignant respiratory diseases in workers of stainless steel and alloyed steel production. Egedahl et al. (2001) study did not show an increase of mortality of respiratory effects in hydrometallurgical workers either. Sorahan (2004) study found a standardized mortality ratio of 69 for respiratory diseases.

6.4.4 Animal data

ECHA concluded that key chronic inhalation toxicity studies in animals for metallic nickel, nickel sulfate, nickel subsulfide and nickel oxide have been conducted by NTP (1996a, b, c), Oller (2008), and Heim (2007). A more comprehensive list of subchronic and chronic toxicity studies can be found in ATSDR (2005) and EFSA (2015; 2020). NTP (1996a, b, c) conducted inhalation exposure of 16 days, 13 weeks, and 2-years for nickel sulfate, nickel subsulfide, and nickel oxide. The main results of these studies are listed in table IV.7. It can be concluded that the main nonneoplastic findings are chronic inflammation in the lungs, lymphoid hyperplasia in the bronchial lymph nodes, and atrophy of the olfactory epithelium (NTP, 1996a, b, c). Neoplastic findings will be discussed in the carcinogenicity chapter. In the 13-week study, chronic inflammation occurred with the same severity at the dose of 0.11 mg Ni/m^3 for nickel sulfate and subsulfide (ECHA, 2018; NTP, 1996b, c). In addition, dose-response slopes were similar. The 2-year study showed a steep dose response for nickel sulfate for chronic lung inflammation and a similar dose-response can be expected for nickel subsulfide based on the 13-weeks study (ECHA, 2018). For chronic inflammation, nickel sulfate has a NOAEC of 0.03 mg Ni/m^3 and a LOAEC of 0.11 mg Ni/m^3. In the NTP studies, no NOAEC was determined in the 2-year studies for nickel subsulfide and nickel oxide (NTP, 1996a, b). Nickel metal caused chronic inflammation of the lungs in a dose dependent manner, with a LOAEL of 0.1 mg Ni/m^3, but no NOAEC could be determined (Oller et al., 2008).

EFSA (EFSA, 2015) concluded that oral repeated-dose toxicity studies show that the most common chronic effects are decreased body weight, increased mortality, changes in organ weights (liver and kidneys), hepatotoxicity, nephrotoxicity, irritation of gastrointestinal tract in rats, and in mice the effect on myeloid system were detected at high doses. Lowest NOAEL 2.2 mg Ni/kg bw was determined in the Heim et al. (2007) study based on a decrease in bodyweight.

No chronic dermal toxicity data was available.

Table IV.9: Key studies of chronic/carcinogenic animal studies

Substance, dose	Model	Main results	Reference
Nickel sulfate hexahydrate 0, 3.5, 7, 15, 30, 60 mg/m³ (0, 0.7, 1.4, 3.1, 6.1, 12.2 mg Ni/m³) 6 h/day, 5 days/week Satellite group: 0, 3.5, 15, 30 mg/m³	Rat F344/N, 16 d, inhalation 5 m and 5 f/dose group satellite group: 4m and 5f/dose group	60 mg/m³: 2m and 5f died 30 mg/m³: 1 f died ↓BW in all doses, ↓BW gain in m, ↑lung weights, lung inflammation, atrophy of the olfactory epithelium occurred in the nasal passages, lymphoid hyperplasia in the bronchial and mediastinal lymph nodes. **NO NOAEC**	(NTP, 1996c)
Nickel sulfate hexahydrate 0, 3.5, 7, 15, 30, 60 mg/m³ (0, 0.7, 1.4, 3.1, 6.1, 12.2 mg Ni/m³) 6 h/day, 5 days/week	Mice B6C3F, 16 d, inhalation 5m and 5f/dose group satellite group: 5m and 5f/dose group	≥7 mg/m³: all m and f mice died ↓BW, ↓BW gain, emaciation, lethargy, rapid respiration, ↑lung weights, lung inflammation in all doses, lung inflammation with necrosis of the alveolar and bronchiolar epithelium at dose 7 mg/m³, atrophy of the olfactory epithelium of the nasal passages at dose 3.5 mg/m³, **NO NOAEC**	(NTP, 1996c)
Nickel sulfate 0, 0.12, 0.25, 0.5, 1, or 2 mg/m³ (0, 0.03, 0.06, 0.11, 0.22, 0.44 mg Ni/m³ 6 h/day, 5 days/week Satellite group: 0, 0.12, 0.5, 2 mg/m³	Rat F344/N, 13 weeks, inhalation 10 m and 10 f/dose group satellite group: 6m and 6f/dose group	2 mg/m³: 1 m died ↑lymphocyte and neutrophil account ≥0.5 mg/m³: lymphoid hyperplasia of the bronchial and/or mediastinal lymph nodes (m), atrophy of the olfactory epithelium ≥0.25 mg/m³: ↑lung weights, atrophy of the olfactory epithelium (f) ≥0.12 mg/m³: Inflammatory lesions of the lungs	(NTP, 1996c)
Nickel sulfate 0, 0.12, 0.25, 0.5, 1, or 2 mg/m³ (0, 0.03, 0.06, 0.11, 0.22, 0.44 mg Ni/m³ 6 h/day, 5 days/week Satellite group: 0, 0.12, 0.5, 2 mg/m³	Mice B6C3F1, 13 weeks, inhalation 10m and 10 f/dose group satellite group: 5-6 m and 5-6 f/dose group	≥2 mg/m³: lymphoid hyperplasia of the bronchial lymph node, atrophy of the olfactory epithelium in the nasal passages ≥1 mg/m³: ↑lung weights, chronic and active inflammation ≥0.5 mg/m³: ↑ alveolar macrophages NOAEC 0.25 mg/m³	(NTP, 1996c)
Nickel sulfate 0, 0.12, 0.25, or 0.5 mg/m³	Rat F344/N, 2-year (104 weeks), inhalation	≥0.5 mg/m³: lower body weight, lymphoid hyperplasia in the bronchial lymph nodes, atrophy of the olfactory epithelium	(NTP, 1996c)

Substance, dose	Model	Main results	Reference
(0, 0.03, 0.06, or 0.11 mg Ni/m³) 6 hours plus T90 (8 minutes) 5 days per week	53-65 m and 63-64 f	≥0.25 mg/m³: chronic active inflammation, macrophage hyperplasia, alveolar proteinosis, and fibrosis ≥0.12 mg/m³: inflammatory lesions in the lungs	
Nickel sulfate 0, 0.25, 0.5, or 1 mg/m³ (0, 0.06, 0.11, or 0.22 mg Ni/m³) 6 hours plus T90 (8 minutes) 5 days per week	Mice B6C3F1, 2-year (104 weeks), inhalation 80 m and 80 f	≥1 mg/m³: lower body weight ≥0.5 mg/m³: lung weight, atrophy of the olfactory epithelium ≥0.25 mg/m³: macrophage hyperplasia, chronic active inflammation, bronchialization (alveolar epithelial hyperplasia), alveolar proteinosis, and infiltrating cells in the interstitium	(NTP, 1996c)
Nickel subsulfide 0, 0.6, 1.2, 2.5, 5, or 10 mg/m³ (0, 0.44, 0.88, 1.83, 3.65, and 7.33 mg Ni/m³) 6 h/day, 5 days/week Satellite group: 0, 0.6, 2.5, or 10 mg/m³	Rat F344/N, 16 d, inhalation 5 m and 5 f/dose group satellite group: 3 m and 3 f/dose group	10 mg/m³: 1 m in died, labored respiration ≥5 mg/m³: ↓ bw, ≥2.5 mg/m³: ↓ bw (f), ↑ lung weights, ≥1.2 mg/m³: ↑absolute lung ≥0.6 mg/m³: inflammation of the lung, atrophy of the nasal olfactory epithelium	(NTP, 1996b)
Nickel subsulfide 0, 0.6, 1.2, 2.5, 5, or 10 mg/m³ (0, 0.44, 0.88, 1.83, 3.65, and 7.33 mg Ni/m³) 6 h/day, 5 days/week Satellite group: 0, 0.6, 2.5, or 10 mg/m3	Mice B6C3F, 16 d, inhalation 5m and 5f/dose group satellite group: 3 m and 3 f/dose group	10 mg/m³: all mice died, labored breathing ≥5 mg/m³: ↓ bw, lung fibrosis ≥2.5 mg/m³: Inflammation of the lung ≥1.2 mg/m³: lymphoid hyperplasia of the bronchial lymph nodes, atrophy of the nasal olfactory epithelium	(NTP, 1996b)
Nickel subsulfide 0, 0.15, 0.3, 0.6, 1.2, 2.5 mg/m³ (0, 0.11, 0.22, 0.44, 0.88, 1.83 mg NI/m³ 6 h/day, 5 days/week Satellite group: 0, 0.15, 0.6, or 2.5 mg/m³	Rat F344/N, 13 weeks, inhalation 10m and 10f/dose group satellite group: 18 m and 18 f/dose group	2.5 mg/m³: ↓ bw (m), labored respiration ≥0.6 mg/m³: atrophy of the nasal olfactory epithelium ≥0.3 mg/m³: Increases in the number of alveolar macrophages, interstitial infiltrates, or incidences of chronic inflammation of the lung ≥0.15 mg/m³: neutrophil and erythrocyte counts, hematocrit values, and hemoglobin concentrations were minimally increased, ↑lung weights, ↑ alveolar macrophages	(NTP, 1996b)
Nickel subsulfide 0, 0.15, 0.3, 0.6, 1.2, 2.5 mg/m³ (0, 0.11, 0.22,	Mice B6C3F, 13 weeks, inhalation 10m and 10f/dose group	≥1.2 mg/m³: ↑lymphocyte counts, lung weights, chronic inflammation and fibrosis, lymphoid hyperplasia of the bronchial lymph nodes	(NTP, 1996b)

Substance, dose	Model	Main results	Reference
0.44, 0.88, 1.83 mg NI/m³ 6 h/day, 5 days/week Satellite group: 0, 0.15, 0.6, or 2.5 mg/m³	satellite group: 6 m and 6 f/dose group	≥0.6 mg/m³: interstitial infiltrates of lymphocytes, atrophy of the nasal olfactory epithelium ≥0.3 mg/m³: ↑hemoglobin concentrations and erythrocyte counts, alveolar macrophages	
Nickel subsulfide 0, 0.15, or 1 mg/m³ (0, 0.11, or 0.73 mg Ni/m³) 6 h/day 5 d/week	Rat F344/N, 2-years (104 weeks), inhalation 63 m and 63 f/dose group	1 mg/m³: ↓ bw, rapid and shallow breathing, ↑hematocrit values and hemoglobin concentrations, ↑erythrocyte count (m), olfactory epithelial atrophy, chronic active inflammation of the nose (f), adrenal medulla hyperplasia (f) ≥0.15 mg/m³: fibrosis; chronic active inflammation; focal alveolar epithelial hyperplasia, macrophage hyperplasia, and proteinosis; bronchial lymphoid hyperplasia; interstitial inflammation, alveolar/bronchiolar adenoma (m), alveolar/bronchiolar carcinoma, alveolar/bronchiolar adenoma or carcinoma (combined), benign pheochromocytoma, malignant pheochromocytoma (m), and combined benign or malignant pheochromocytoma (m), lymphoid hyperplasia of the bronchial lymph node	(NTP, 1996b)
Nickel subsulfide 0, 0.6, 1.2 mg/m³ (0, 0.44, 0.88 mg Ni/m³) 6 h/day 5 d/week	Mice B6C3F, 2-years (105 weeks), inhalation 80 m and 80 f/dose group	≥0.6 mg/m³: lung weight, chronic active inflammation, bronchialization (alveolar epithelial hyperplasia), macrophage hyperplasia and proteinosis, interstitial infiltration, fibrosis, atrophy of the nasal olfactory epithelium and inflammation of the nose, lymphoid hyperplasia of the bronchial lymph node, macrophage hyperplasia, ↓ degeneration of olfactory epithelium (f), ↓alveolar/bronchiolar carcinoma (f), ↓alveolar/bronchiolar adenoma or carcinoma (combined)	(NTP, 1996b)
Nickel oxide 0, 1.2, 2.5, 5, 10, or 30 mg/m³ (0, 0.9, 2.0, 3.9, 7.9, or 23.6 mg/m³) Satellite group: 0, 1.2, 5, or 10 mg/m³ 6 h/day 5 d/week	Rat F344/N, 16 d, inhalation 5 m and 5 f/dose group satellite group: 5 m and 5 f/dose group	≥30mg/m³: Hyperplasia of bronchial lymph nodes, atrophy of the olfactory epithelium in one m and f ≥10mg/m³: lung weights, Chronic-active inflammation and accumulation of macrophages in alveolar spaces of the lungs and hyperplasia in the respiratory tract lymph nodes ≥1.2mg/m³: pigment particles in alveolar macrophages or within the alveolar spaces	(NTP, 1996a)

Substance, dose	Model	Main results	Reference
Nickel oxide 0, 1.2, 2.5, 5, 10, or 30 mg/m³ (0, 0.9, 2.0, 3.9, 7.9, or 23.6 mg/m³) Satellite group: 0, 1.2, 5, or 10 mg/m³ 6 h/day 5 d/week	Mice B6C3F1, 16 d, inhalation 5 m and 5 f/dose group satellite group: 5 m and 5 f/dose group	≥2.5mg/m³: pigment in the lungs, accumulation of macrophages in alveolar spaces	(NTP, 1996a)
Nickel oxide 0, 1.2, 2.5, 5, 10 mg/m³ (0, 0.9, 2.0, 3.9, 7.9 mg/m³) Satellite group: 0, 0.6, 2.5, 10 mg/m³ 6 h/day 5 d/week	Rat F344/N, 13 weeks, inhalation 10 m and 10 f/dose group satellite group: 5 m and 5 f/dose group	Lung weight, ↑Lymphocyte, neutrophil, monocyte, and erythrocyte counts; hematocrit values; and hemoglobin and mean cell hemoglobin concentrations ≥2.5mg/m³: nonneoplastic lesions in the lungs, inflammation in the lungs, lymphoid hyperplasia and pigment in the bronchial and mediastinal lymph nodes ≥1.2mg/m³: accumulation of alveolar macrophages, many of which contained black, granular pigment	(NTP, 1996a)
Nickel oxide 0, 1.2, 2.5, 5, 10 mg/m³ (0, 0.9, 2.0, 3.9, 7.9 mg/m³) Satellite group: 0, 0.6, 2.5, 10 mg/m³ 6 h/day 5 d/week	Mice B6C3F1, 13 weeks, inhalation 10m and 10f/dose group satellite group: 6 m and 6 f/dose group	10 mg/m³: ↑lung weights, ↓liver weights (m) ≥5 mg/m³: ↑hematocrit values, erythrocyte counts and hemoglobin concentration (f) ≥2.5 mg/m³: inflammation, lymphoid hyperplasia, pigment in the bronchial lymph nodes ≥1.2 mg/m³: accumulation of alveolar macrophages, many of which contained pigment particles	(NTP, 1996a)
Nickel oxide 0, 0.62, 1.25, or 2.5 mg/m³ (0, 0.5, 1.0, 2.0 mg Ni/m³ 6 h/day 5 d/week	Rat F344/N, 2-years (104 weeks), inhalation 65 m and 65 f/dose group	≥2.5 mg/m³: ↓bw, benign pheochromocytoma(f), adrenal medulla hyperplasia (f), benign or malignant pheochromocytoma (combined) (m) alveolar/bronchiolar adenomas alveolar/bronchiolar adenoma or carcinoma (combined), ≥1.25 mg/m³: ↓bw(f), ↑lung weights, pigmentation in the bronchial lymph nodes similar to that in the lungs, ≥0.62 mg/m³: pigmentation in the alveolus, atypical alveolar epithelial hyperplasia in the lungs, chronic inflammation of the lung	(NTP, 1996a)
Nickel oxide	Mice B6C3F1, 2-years (104 weeks),	5mg/m²: ↓bw, 2.5 mg/m²: alveolar/bronchiolar adenoma (f)	(NTP, 1996a)

Substance, dose	Model	Main results	Reference
0, 0.62, 1.25, 2.5, 5 mg/m³ (0, 0.5, 1.0, 2.0,3.9 mg Ni/m³ 6 h/day 5 d/week	inhalation74-79 m and 74-79 f/dose group	≥1.25mg/m²: alveolar/bronchiolar adenoma or carcinoma (combined), chronic inflammation, alveolar epithelial hyperplasia, and proteinosis, pigment occurred in the lungs, lymphoid hyperplasia, pigmentation in the bronchial lymph nodes	
Nickel metal 0, 0.1, 0.4, and 1.0 mg Ni/m³ 6 h/d, 5 d/week	Rat Wistar, 2-years (104 weeks) 50 m and 50 f/dose group	1mg/m³: Mice were killed at 12 m due to increased mortality. ↓bw, ↓food consumption 0.4 mg/m³: ↓bw, ↓food consumption, increased mortality at 18 m (f) and exposure stopped at this point. Increased respiratory rate, cyanosis of extremities, cool/pale extremities and/or body, dermal atonia, thinness, decreased defecation, and labored respiration, ≥0.1 mg/m³: ↓bw (m), ↑red blood cell counts, ↑hemoglobin levels, ↑hematocrit values, lung weight, alveolar proteinosis, alveolar histiocytosis, chronic inflammation, chronic-active inflammation, bronchiolar-alveolar hyperplasia, pigment in the kidneys, extramedullary hematopoiesis in the spleen, and hypercellularity of the sternum and femoral bone marrows, combined adenoma/carcinoma of the adrenal cortex (f), pheochromocytomas of the adrenal medulla (m)	(Oller et al., 2008)
Nickel sulfate hexahydrate 0, 10, 30, 50 mg/kg bw daily (0, 2.2, 6.7, 15.6 mg Ni/kg bw)	Rat F344N, oral gavage, 2-years 60 m and 60 f/dose group	≥ 50mg/kg: ↑eosinophils, bronchial inflammation secondary due to oral gavage ≥30 mg/kg: ↓bw, ↑red blood cells (m), ↑hematocrit(f), ↑hemoglobin(f), ↓leukocytes (f) ≥10mg/kg: increased mortality (f), ↑leukocytes(f),	(Heim et al., 2007)

m=males, f=females

6.5 Reproductive and developmental toxicity

6.5.1 Human data

ECHA (2018) and EFSA (2015) concluded that no reproductive and developmental effects have been seen in the epidemiological studies after inhalation or oral exposure to nickel. However, EFSA (2020) concluded in its latest report that there may be an association between nickel exposure and reproductive and developmental toxicity. Epidemiological studies suggest that nickel exposure may decrease sperm concentration and mobility and increase the risk of pre-term delivery, congenital heart defects, and orofacial clefts. Since then, a few additional studies have been published suggesting that nickel exposure may affect birth weight, neurocognitive development, hormonal imbalance, and semen quality. Here, more details of these studies are presented.

In the cohort study, the dose-response relationship between blood nickel levels and testosterone (in women, n=218), estradiol and progesterone (in men, n=217) was studied, and BMD was calculated (Marić et al., 2022). However, the BMDU/BMDL ratio was wide for all the hormones (testosterone 1 640000/0.000235, estradiol 63300/0.000126, progesterone 215/0.00000202), thus, the study results are not reliable.

In the cross-sectional occupational study in Egypt, male electroplaters (n=105) exposed to soluble nickel compounds was compared into controls (n=60) (Beshir et al., 2016). Mean urine nickel concentration was 4.18 µg/g creatinine in exposed workers and 1.67 µg/g creatinine in controls. Other exposure substances (metals or mist suppressants) or confounding factors (alcohol, cigarette smoke) were not controlled. The exposed workers had higher serum luteinizing hormone and follicle stimulating hormone levels compared to controls. Testosterone levels did not differ from controls. In addition, the incidence of more than one sexual disorder was increased in exposed workers when compared to controls. The odds ratio for workers versus controls for decreased libido was 9.5 (95 % CI 2.78-32.5), for impotence OR was 5.1 (95 % CI 2.01-12.98) and for premature ejaculation OR was 4.4 (95 % CI 1.97-9.9). However, the study had limitations related to the study size and to the control of confounding factors.

Howe et al. (2022) conducted a pooled analysis from three cohorts (MADRES, NHBCS, and PROTECT, total n=1002) to study metal mixtures and birth weight for gestational age. The mean urine nickel concentration was 4.6 ± 5.1 µg/L. They found a positive association between urine nickel concentration and birth weight for gestational age, but there was variability among the cohorts. The researchers hold all other metals in the mixture constant at their median and adjusted the data for maternal age, pre-pregnancy BMI, maternal education, parity, gestational age at urine collection, and in utero tobacco smoke exposure.

In the follow-up study, an association between preterm nickel exposure and childhood allergic diseases (allergic rhinitis, wheeze and eczema) was studied (Ruan et al., 2022). Urine nickel concentrations during pregnancy were measured, and children were followed up until they were 4-years old. The median urine concentration for the trimester average was 3.53 µg/L. In the linear regression analysis, nickel alone did not show an association with allergic rhinitis, wheeze, or eczema. However, a combined effect with vanadium, chromium, nickel, arsenic, cadmium, thallium, and lead showed an association with allergic rhinitis (adjusted for other metals).

In a Chinese cohort (n=703), the association between preterm exposure to metals and the neurocognitive development of children was studied (Liu et al., 2022). Maternal serum nickel was measured. The mean age of the followed-up children was 2.57±0.14. Nickel postpartum metal concentration from infants was measured from urine. The median concentration of infant urine was 0.76 µg/mmol creatinine. Nickel postpartum exposure was negatively associated with adaption in the single-metal model and in multiple-metal model. Models were adjusted for maternal age, pre-pregnancy BMI, children's age, children's gender, blood sampling time, delivery mode, delivery gestational week, and birth head circumference.

The association between nickel blood concentration and semen quality was studied in males (n=179) (Calogero et al., 2021). An increased blood nickel concentration was detected in men with abnormal sperm concentration (12.30 vs. 7.69 µg/L), total sperm count (19.07 vs. 7.04 µg/L), and sperm progressive motility (14.50 vs. 2.75 µg/L) when compared to men with normal sperm quality. There were no differences in the seminal plasma nickel concentration of men with normal sperm parameters or oligozoospermia/asthenozoospermia. The nickel concentration of sperm (0.088 vs. 0.040 µg/L) was higher in men with a total sperm count of < 39 mil/ejaculate than in men with a total sperm count of > 39 mil/ejaculate.

6.5.2 Animal data

EFSA (2015; 2020) provided an extensive review of the available reproductive and developmental toxicity animal studies. They concluded that nickel could cause alterations in male sex organ weights, histopathological changes in these organs, disturbed spermatogenesis, decreased sperm motility, and sperm damage in mice resulting in decreased fertility. In addition, there is some evidence that nickel may damage the testes of the rats. Mice seem to be more sensitive than rats due to protamine 2 expression in the sperm

chromatin; rats have only a little of protamine 2 (EFSA, 2020). Protamine 2 is involved in the reactive oxygen species (ROS) production, leading to an increased ROS in the testes. Humans have protamine 2, thus these effects on fertility are likely to be relevant for humans too (EFSA, 2020). In females, nickel exposure has resulted in irregular ovarian cycle, inhibition of ovulation, and a decrease in implantation frequency (EFSA, 2020). Zhao et al. (2021) studied that nickel sulfate causes ROS, and DNA damage, and moreover, inflammation and fibrosis of oocytes leading to functional disorders in oocyte mitochondria, resulting in decreased oocyte quality due to disturbed ovarian homeostasis and follicle development.

Developmental toxicity studies show that nickel can cause increased pup mortality, decreased pup weight, and malformations in rats and mice (EFSA, 2020). In rats, toxicity occurs with lower doses than in mice. EFSA conducted benchmark dose modelling for developmental toxicity based on SLI (2000a, b) one and two-generation studies resulting in a BMDL10 of 1.3 mg/kg bw. BMDL10 represents 10% extra risk for the background risk. Here, more details of the key studies will be presented, along with the studies published since the EFSA (2020) report.

SLI (2000a) performed a one-generation reproduction range-finding study on Sprague-Dawley rats with nickel sulfate hexahydrate. F0 female and male rats received nickel sulfate hexahydrate (0, 2.2, 4.4, 6.6, 11, and 17 mg Ni/kg bw per day) by oral gavage, starting 2 weeks prior to mating and continuing throughout gestation. The study showed an increase in post-implantation loss in the offspring at doses ≥6.6 mg/kg bw per day, increased dead pups at all doses, and a decreased litter size at dose level 17 mg Ni/kg bw per day. The surviving pups grew normally and no changes in the histopathology were detected. The NOAEL for parental toxicity was 17 mg Ni/kg bw per day and the LOAEL for offspring was 2.2 mg/kg bw per day.

SLI (2000b) conducted a two-generation reproduction toxicity study on Sprague-Dawley rats with nickel sulfate hexahydrate (0, 0.2, 0.6, 1.1, and 2.2 mg Ni/kg bw per day) exposure via oral gavage. In the F1 generation, the mean post-implantation loss was higher at the dose of 2.2 mg Ni/kg bw per day, but it did not reach statistical significance and remained within the historical range. In the F2 generation, similar increase was not detected. No changes in the pups´ viability or growth were detected at any of the dose levels in either the F1 or F2 generation. The NOAEL was determined to be 2.2 mg Ni/kg bw per day.

Since the publication of the EFSA report (2020), several studies have been published, and most of them studied nickel nanoparticles. The main results from these studies are listed in table IV.8. Nickel nanoparticles show effects on sperm motility and histopathological changes in testes in a dose-dependent manner (Hu et al., 2020; Iftikhar et al., 2023; Kong et al., 2021). The route of exposure varied between the studies. The most relevant studies are oral/inhalation exposure studies, as they mimic the physiological exposure route, thus, being the most important for determining dose-response. Most of the nickel nanoparticle studies included a nickel microparticle group at the dose level of 45 mg/kg that produced similar or more serious changes than nickel nanoparticles. In addition, Singh et al. (2022a; 2022b) studied nickel oxide nanoparticles in Wistar rats, both females and males. They found similar findings in males than studies with nickel nanoparticles (Singh et al., 2022b). In females, nickel oxide nanoparticles decreased the number of primordial, primary, and secondary follicles, caused hyperplasia of Graafian and atretic follicles, constriction of the ovarian cortex and medulla, and proliferation of the corpus luteum (Singh et al., 2022a). Hu et al. (2020) performed a 30-day study with oral gavage on ICR mice but they only studied male reproductive organs. No NOAEL could be determined, the LOAEL was 5 mg/kg/day for nickel nanoparticles.

Table IV.10: Reproductive toxicity studies published after EFSA (2020) report

Substance, dose	Model	Main results	Reference
Nickel nanoparticles (NI NP), mean size 90 nm 0, 5, 15, 45 mg/kg/day	ICR mice, male, 30 d, oral gavage, 60 mice divided into 5 groups	Ni MP: ↓body weight ↑Epididymis and testis somatic index, sperm motility. No histological data was presented.	(Hu et al., 2020)

Substance, dose	Model	Main results	Reference
Nickel microparticles (Ni MP), 3 µm, 45 mg/kg/day		≥5 mg/kg Ni NP: ↑Epididymis and testis somatic index, sperm motility, apoptosis and ↓ number of spermatocytes, ↑testis marker enzyme AKP and ACP	
Nickel sulfate 0, 5, 10, 20 mg/kg/d	ICR mice, female, 21 d, i.p., 60/dose group Study focused on ovaries	20mg/kg: ↓ number of preovulatory follicle, ↑double strand breaks, ↓histone methylation ≥10 mg/kg: ↑number of atretic follicles, fibrosis, ↑ROS ≥5mg/kg: ↓ bw growth, ↓ ovary weight and size, ↑ DNA breaks, inflammation, ↑fibronectin, ↓oocyte development, early apoptosis, altered mitochondrial morphology and function	(Zhao et al., 2021)
Nickel oxide nanoparticles (NIONP), size <30nm, 0, 5 mg/kg bw/d nickel oxide microparticles (NIOMP), 5 mg/kg bw/d	Wistar rats, oral gavage, 30 d, 10/dose group	NIONP: ↓sperm count, ↓motility, ↑epididymis weight, ↑vas deferens weight, ↑seminal vesicle, sperm abnormalities (more severe than in NIOMP group), DNA damage (more severe than in NIOMP group), oxidative stress, histopathological changes in testis, epididymis, vas deferens, prostate gland and seminal vesicles NIOMP: ↓sperm count, ↓motility, ↓testes weight, ↑vas deferens weight, ↓seminal vesicle, sperm abnormalities, DNA damage, oxidative stress, histopathological changes in testis, epididymis, vas deferens, prostate gland and seminal vesicles	(Singh et al., 2022b)
Nickel oxide nanoparticles (NIONP), size <30nm, 0, 5 mg/kg bw/d nickel oxide microparticles (NIOMP), 5 mg/kg bw/d	Wistar rats, female, oral gavage, 15 d and 30 d, 5/dose group	Nickel accumulated in the ovaries, nanoparticles more than microparticles. ↑ progesterone and estradiol when compared to control. ROS was generated in NIONP and NIOMP groups but in a greater manner at NIONP group. ↑Caspace-3 at NIONP and NIOMP group but more in NIOMP group (apoptosis marker). NIONP: ↓the number of primordial, primary, and secondary follicles; hyperplasia of Graafian and atretic follicles, constriction of cortex and medulla, proliferation of corpus luteum. NIOMP: enlargement of primordial and primary follicles but constriction of secondary follicles, proliferation of theca externa and theca interna of Graafian follicles, atrophy of cortex and medulla, proliferation of corpus luteum. NIONP and NIOMP: changes in mitochondrial shape and size, increased number of polyribosomes, disruption of granulose layer of follicles.	(Singh et al., 2022a)
Nickel nanoparticles, 90 nm 0, 5, 15, 45 mg/kg/week	BALB/c mice, male, intratracheal, 28 d,	all exposed groups except 15 mg/kg: ↓testosterone; ↓follicle-stimulating hormone; ↓luteinizing hormone, ↑TNF-α, Il-6, IL-1β	(Kong et al., 2021)

Substance, dose	Model	Main results	Reference
nickel microparticles, 3 µm, 45 mg/kg/week Nickel chloride, 45 mg/kg/week	10/dose group	all exposed groups: sperm malformation, pathological changes in testes, upregulation of mitophagy signaling pathway PINK1/Parkin 45 mg/kg NiNP, NiMP, NiC2: ↑ apoptosis of testicular tissue,	
Nickel nanoparticles, 56 nm, placebo (0.9% saline), 0, 15, 30, 45 mg/kg	Sprague Dawley rats, i.p., 14 d, 5/dose group	45 mg/kg: accumulation of Ni in testes, sloughing, necrosis, wide lumen with absent sperm cells and coagulative necrosis ≥30 mg/kg: ↓bw, in the testis: necrosis, loss of spermatogenic series along with vacuation, spectrum of germ cell absence, mineralization ≥15 mg/kg: testosomatic index, ↓testosterone levels, ↓ sperm production, Histological changes in testes: basement membrane damage, vacuation, necrosis with vacuole formation, mineralization inside and outside the seminiferous tubule	(Iftikhar et al., 2023)

d=day

6.6 Neurotoxicity

EFSA (2020) found a few studies on neurotoxicity in vivo. Studies suggest that water-soluble nickel compounds may affect neurobehavioral functions in rodents, as detected by impaired spatial memory performance, locomotor hyperactivity and neurodegeneration in adult rats. Since then, an in vivo study in mice has provided further support that nickel chloride (10mg Ni/L in drinking water for 12 weeks) may impair memory and learning (Zhou et al., 2021).

In humans, only a few studies are available on the association between nickel exposure and neurotoxicity. The results of these studies are inconsistent (EFSA, 2020). The association between nickel in spinal fluid and the risk of amyotrophic lateral sclerosis (ALS) was studied (n=38) (Chen et al., 2022). Nickel levels were higher in the spinal onset group (4.21 µg Ni/L) than in the bulbar-onset group (3.30 µg Ni/L, P=0.03) or in the control group (2.23 µg Ni/L, P=0.02). However, nickel did not correlate with the severity of ALS. In addition, in another study, no differences were detected between spinal fluid nickel concentrations and bulbar-onset or spinal-onset group (Patti et al., 2020).

6.7 Endocrine disruption

6.7.1 Thyroid hormone effects

There is limited evidence on thyroid hormone effects of nickel. In a recent study, the relationship between nickel blood concentration and serum thyroid hormone levels and parameters of thyroid homeostasis (SPINA-GT and SPINA-GD) were studied in cancer patients to analyze whether nickel may disrupt thyroid function (Maric et al., 2023). The study population included healthy volunteers (n=136), prostate/testicular cancer patients (n=104), breast cancer and benign breast dysplasia patients (n=96), pancreatic cancer patients (n=22), and thyroid and metabolic disorders patients (n=77). The study found a correlation between male blood nickel concentration and fT4 and SPINA-GT. No correlations were detected in females or in the whole study population. The study had limitations related to study set up, sample size, and control of confounding factors. No conclusions of causality can be drawn based on this cross-sectional study.

The case-control study (n=110) that determined a correlation between blood Ni and hypothyroidism, hyperthyroidism, and thyroid cancer did not find a correlation (Rezaei et al., 2019). Also, study in pregnant Chinese women (n=915) did not find significant correlation between blood nickel concentration and serum levels of thyroid hormones (Guo et al., 2018). The retrospective study (n=260) found an increased risk of chronic autoimmune thyroiditis in SNAS patients when compared to non-SNAS patients. The non-SNAS patients study group were compiled from patients with other immune-mediated inflammatory disease other than SNAS (Andrioli et al., 2015). No causality can be drawn based on the study.

In animals, i.p administration of nickel sulfate 2.5, 5 and 10 mg/kg day, for 40 days) has resulted in decrease of serum concentration of fT4 and TSH in male rats at all dose levels (Liu et al., 2020). Direct damage of thyroid cells and vascular congestion was detected in the highest dose group. ATSDR (2024) concluded that no histological changes were detected in rats or mice adrenal glands, pancreas, parathyroid, pituitary, or thyroid glands following exposure of nickel sulfate, nickel oxide, or nickel subsulfide for 12 days (6-hour exposure) over 16 days or for 6 hours/day, 5 days/week for 13 weeks. The NOAEL for endocrine effects for nickel sulfate was 0.44 mg Ni/m^3, for nickel oxide 7.9 mg Ni/m^3, and for nickel subsulfide 1.83 mg Ni/m^3 based on 13-weeks study.

6.7.2 Other endocrine effects

ATSDR reported one human study showing changes in cortisol levels (ATSDR, 2024). Nickel urine and cortisol concentrations were measured twice one year apart in the shipyard workers (n=49) and controls (n=20) in Taiwan (Lai et al., 2021). The study detected a significant association between urine nickel concentration and decreased urine cortisol (β = -0.161 for a 1-µg/g increase in creatinine-adjusted urine nickel concentration) with a linear mixed-effect model after adjusting for the smoking status, working type, and age. The authors suggested cortisol insufficiency could be associated with adrenal gland dysfunction.

In animals, no histological alterations were detected in the endocrine glands of rats exposed to nickel sulfate of 2.2 mg Ni/kg/day for 16 weeks or 22 mg Ni/kg/day for 90 days (ATSDR, 2024). In addition, nickel sulfate exposure through diet in rats (187.5 mg Ni/kg/day/2 years) and dogs (62.5 mg Ni/kg/day/2 years) did not show histological alterations in endocrine glands.

As stated earlier in the chronic toxicity section nickel has also been associated with diabetes and metabolic diseases (Titcomb et al., 2021; Wang et al., 2023). In animals, changes in serum glucose levels have been observed in rats exposed to airborne nickel oxide 23.6 hours/day for 21–28 days (ATSDR, 2024). In females, no clear dose-response was detected. In male rats, increased serum glucose levels were detected at dose level of 0.385 mg Ni/m3. ATSDR (2024) also reported another study that showed decreased blood glucose levels in female rats exposed to nickel chloride at the dose level of 8.6 mg Ni/kg/day 91 days.

As a conclusion, only a limited amount of evidence is available of endocrine effects and nickel especially in humans and further studies are required to make conclusions. Thus, currently endocrine effects cannot be used as a basis of an HBM-GV derivation.

6.8 Immunotoxicity

Immunological effects of nickel include contact dermatitis that has been thoroughly described earlier in the skin sensitization chapter (ATSDR, 2024). In addition, impaired immune function and histological alterations have been detected in animals following nickel exposure (ATSDR, 2024). Inhalation exposure to nickel sulfate, nickel subsulfide, and nickel oxide in rats and mice, have resulted in lymphoid hyperplasia in the bronchial and mediastinal lymph nodes (ATSDR, 2024). Additionally, metallic nickel exposure has led to histiocyte infiltrates in lymph nodes in rats. Animals exposed via inhalation were more susceptible to bacteria and had impaired response to sheep red blood cells (sRBCs). Similar impairment of immune responses has been detected following oral exposure to nickel chloride and nickel sulfate. In addition, nickel sulfate exposure in rats has shown histological changes in spleen and thymus T cell phenotypes (ATSDR, 2024). Additionally, a recent review concluded that excessive nickel exposure may inhibit the development of immune organs by inducing apoptosis and inhibiting proliferation (Guo et al., 2020). Authors also concluded that nickel inhibits production of cytokines in non-inflammatory responses.

Acute immunotoxicity effects (including impaired immune function and lymphoid hyperplasia in bronchial lymph nodes) have been detected at concentrations above 0.25 mg Ni/m^3 in rodents (ATSDR, 2024). Sub-chronic toxicity studies resulted NOAEL values ranging between 0.025-0.22 mg Ni/m^3, and LOAEL values between 0.145-9.2 mg Ni/m^3 depending on the nickel species and animal species (ATSDR, 2024). The immunological effects included lymphoid hyperplasia in the bronchial and mediastinal lymph nodes and altered impaired immune function. The chronic toxicity (lymphoid hyperplasia in bronchial lymph nodes) has been detected at concentrations above 0.1 mg Ni/m^3 (ATSDR, 2024).

6.9 Genotoxicity

The genotoxic effects of nickel have been widely studied and reported by ECHA (2018) and EFSA (2015; 2020). Here, main findings are summarized.

6.9.1 In vitro

Nickel compounds have been negative in bacterial mutagenicity tests and showed only weak positivity in cultured mammalian cell mutagenicity tests (EFSA, 2020). In Chinese hamster V97 cells, soluble nickel chloride slightly increased mutations in the hypoxanthine-guanine phosphoribosyltransferase (*hprt*) gene detected by increased resistance to 8-azaguanine at cytotoxic doses (Miyaki et al., 1979). In addition, in the *hprt* gene model in Chinese hamster V97 cells and xanthine-guanine phosphoribosyl transferase transgene (*gpt*) model in G10 and G12 cell lines insoluble nickel compounds (nickel sulfide,-subsulfide, -oxides) and nickel chloride have been studied (Kargacin et al., 1993). The only positive response was in the G12 cell line. Vitamin E increased the cell survival of the insoluble compounds, indicating the role of oxidative damage. Lee et al. (1995) showed that nickel induces DNA methylation, silencing the *gpt* gene. In addition, condensed chromatin and heterochromatinization of the gpt integration site in 6-thioguanine-resistant cells were shown. These results suggest that nickel carcinogenicity is due to an epigenetic mechanism instead of mutagenicity. It should be noted that studies indicate nickel to have a co-mutagenicity effect with for example alkylating agents and ultraviolet light most likely due to interference with DNA repair processes (EFSA, 2015). In addition, nickel metal powder was studied in V79 Chinese hamster cells according to OECD guidelines 476 and 487 (Buxton et al., 2020). Nickel metal was neither mutagenic nor and did it cause gene or chromosomal mutations.

DNA strand breaks were induced by nickel chloride and crystalline nickel sulfide in cultured Chinese hamster ovary cells (Robison and Costa, 1982). Nackerdien et al. (1991) showed that nickel (II) ions, in the presence of hydrogen peroxide, induce changes in DNA in chromatin more than in isolated DNA. They identified hydroxyl-radical induced products of DNA bases and confirmed these changes with partial inhibition using scavengers of hydroxyl radicals. The ability of nickel compounds to produce oxidative DNA damage has been confirmed since then (Inoue and Kawanishi, 1989; Kawanishi et al., 2001; Kawanishi et al., 1989, 1994; Kawanishi et al., 2002). They found that in the HeLa and Raji (cultured human) cells, DNA strand breaks

(single and double) and 8-hydroxydeoxyguanosine (8-OH-dG, a biomarker of oxidative DNA damage) were induced by nickel subsulfide but not with nickel oxide or nickel sulfate (Kawanishi et al., 2001). However, in Wistar rat lungs all these compounds induced 8-OH-dG production. In addition, nitric oxide was produced in the macrophage cell line RAW 246.7 after co-culturing each of the nickel compounds with lipopolysaccharide. Two possible mechanisms have been identified 1) indirect oxidative DNA damage through inflammation, and 2) direct oxidative DNA damage through hydrogen peroxide formation (Kawanishi et al., 2001; Kawanishi et al., 2002). The direct mechanism was mainly related to nickel subsulfide. Reactive oxygen species (ROS) production has also been linked to DNA protein crosslinks (EFSA, 2015). Nickel subsulfide caused dose-dependently DNA protein crosslinks due to ROS formation in rat lymphocytes. This process was most likely started with Fenton/Haber-Weiss reaction because an iron chelator, a deferoxamine, inhibited DNA protein crosslink production (Chakrabarti et al., 2001). Nickel compounds (nickel sulfate, nickel subsulfide, nickel oxide, and nickel carbonate hexahydrate) similarly caused single strand breaks in chromosomal and nuclear chromatin due to iron-mediated ROS formation in human blood lymphocytes (M'Bemba-Meka et al., 2005). In addition, nickel compounds disturbed cellular calcium homeostasis, causing single strand breaks (M'Bemba-Meka et al., 2005). Nickel chloride and nickel oxide induced single strand breaks and oxidative damage in the A549 human lung cell line (Schwerdtle and Hartwig, 2006). The clearest effects for both compounds were detected when a long treatment time (20-24 hours) and a cytotoxic dose were used. Additionally, nickel oxide reached the nucleus with a higher fraction than nickel chloride. The authors suggest that carcinogenicity potential for nickel oxide could be due to a long retention time as after inhalation exposure, due to impaired clearance, and higher persistence in the lungs when compared to water soluble compounds, leading to an impaired DNA repair system. Soluble nickel compounds have been confirmed to cause DNA single strand breaks at the highest dose level (Belliardo et al., 2018; Lou et al., 2013). In addition, nickel induced double strand breaks have been studied. Kopp et al. (2018) did not find nickel chloride induced double strand breaks in human hepatoblastoma Hep G2 and colorectal adenocarcinoma LS-174T cells measured by the γ-H2AX assay. Scanlon et al. (2017) studied nickel chloride induced double strand breaks in A549 human lung carcinoma and BEAS-2B non-tumorigenic cells and found a dose-response in double strand breaks.

Several chromosomal effects have been detected by nickel compounds: chromosomal aberrations, sister chromatid exchanges and micronuclei (EFSA, 2015; EFSA, 2020). The aneugenic and clastogenic effects of nickel chloride and nickel sulfate were studied using the kinetochore-stained micronucleus test in human diploid fibroblasts (Seoane and Dulout, 2001). Both compounds tested positive for both aneugenic and clastogenic effects, although, the aneugenic response was stronger. In addition, nickel sulfate showed chromosomal instability (ring and dicentric chromosomes) and aneuploidy in V79 Chinese hamster cells (Ohshima, 2003). Nickel sulfide and nickel chloride have shown ability to cause chromosomal aberrations in a dose-dependent manner (Sen et al., 1987; Sen and Costa, 1985). Especially, chromatid exchange at heterochromatic regions was detected. Furthermore, there are studies showing that both soluble and poorly soluble compounds can cause cell transformations, but poorly soluble compounds are more potent in causing cell alterations (Costa et al., 1982)

In addition, the genotoxicity of nickel, nickel oxide nanoparticles, and nickel chloride have been studied in human lung cells. They have caused DNA strand breaks in comet assay, but no induction on micronucleus (Gliga et al., 2020). However, in THp-1 cells, nickel oxide nanoparticles did induce micronuclei (Vallabani and Karlsson, 2022).

6.9.2 In vivo

In vivo mutation studies have been only weakly positive, and studies have been conducted mainly in *Drosophila melanogaster* (EFSA, 2015). After inhalation of nickel subsulfide (4-13 mg Ni_3S_2/kg bw), *Lack Z* transgenic CD2F1 mice and *Lac I* rats did not show mutagenicity in the nasal or lung tissue (Mayer et al., 1998). However, in mice nasal mucosa DNA strand breaks were detected by a comet assay after the nickel subsulfide exposure.

DNA breaks and DNA-protein crosslinks have been detected after in vivo exposure to both insoluble and soluble nickel compounds (ECHA, 2018). Nickel sulfate and nickel subsulfide caused DNA-strand breaks, detected by the Comet assay, in the lung after 3- or 13-week inhalation exposure (Benson et al., 2002; ECHA, 2018). Nickel sulfate had a NOAEL of 0.04 mg/m^3; and a LOAEL of 0.11 mg/m^3, and nickel subsulfide had NOAEL of 0.04 mg/m^3 and a LOAEL of 0.11 mg/m^3. In addition, inflammation was detected at the same levels as DNA strand breaks. Also, after a single oral exposure of nickel chloride dose-dependent increase in single and double strand breaks in mice leukocytes were detected using the Comet assay (LOAEL 3.4 mg/kg) (Danadevi et al., 2004b). Oxidative DNA damage was studied by single 1 mg intratracheal instillation of nickel subsulfide, nickel oxide (black and green), and nickel sulfate (Kawanishi et al., 2002). An increase of 8-OH-dG was observed in the lungs of rats, with nickel subsulfide causing the highest response, followed by nickel oxide, and then nickel sulfate. The authors suggest two mechanisms for oxidative DNA damage: nickel compounds mostly cause indirect oxidative DNA damage via inflammation but nickel subsulfide can also cause direct oxidative damage through hydrogen peroxide. In addition, Saplakoglu et al. detected single-strand breaks in rat kidney and lung, but not in the liver, after s.c administration of nickel chloride (44 mg/kg bw) (Saplakoğlu et al., 1997).

In the NTP studies (1996a, b), nickel oxide (dose 0-5.0 mg/m^3) and nickel subsulfide (0-1.2 mg/m^3) did not induce an increase in the frequency of micronucleated erythrocytes in peripheral blood samples after 13 weeks inhalation exposure of male or female mice. An *In vivo* micronucleus test was not conducted for nickel sulfate, but *in vitro* mouse lymphoma assay was positive at a dose level of 500 µg/ml and above (NTP, 1996c). No clastogenic effect was found in male mice after i.p. exposure to nickel chloride or nickel dinitrate (Deknudt and Léonard, 1982). Oller and Erexson (2007) studied nickel sulfate hexahydrate in Sprague-Dawley rats. Oral exposure to nickel sulfate did not induce micronucleated polychromatic erythrocytes (PCEs) at any of the studied dose levels (125-500 mg/kg bw/day). In another study, nickel chloride (72.2 mg/kg bw), nickel nitrate (73 mg/kg bw), and nickel sulfate (95 mg/kg bw) induced micronuclei and sperm abnormalities after single oral exposure in mice (Sobti and Gill, 1989). This study was not conducted according to guidelines and no information was available on the number of animals or the purity of test substance. However, positive findings have been found in other studies. Nickel chloride (s.c. 40, 80, and 120 µmol/kg bw/day for 3 days) induced incidence of micronucleated PCEs, chromosomal aberrations, and single-strand breaks (El-Habit and Abdel Moneim, 2014). Dhir et al. showed i.p. exposure to nickel chloride causing chromosomal aberrations and micronuclei (Dhir et al., 1991). Overall, positive micronuclei studies are older and do not follow guidelines and the exposure route has been other than physiological. It can be concluded that physiologically relevant inhalation and oral micronucleus studies have been negative.

Efremenko et al. (2014) studied the mode-of-action of carcinogenesis after exposure to nickel subsulfide (0.0, 0.04, 0.08, 0.15, and 0.60 mg/m^3 (0.03, 0.06, 0.11, and 0.44 mg Ni/m^3) in Fischer 344 rats for one and four weeks (6h/day, 5 days/week). The evaluation of broncho-alveolar lavage fluid and histopathology showed inflammatory effects at the two highest doses, indicating a NOAEL of 0.08 mg/m^3 (0.06 mg Ni/m^3). These inflammation changes were similar to those observed in NTP (1996b) study (table IV.7). The pathway enrichment study detected cell-signaling responses to toxicity, especially in inflammatory and proliferative signaling pathways. In addition, BMD analysis from the gene expression pathways was conducted. The lower values of BMD range for inflammation was 0.06 mg Ni/m^3, for oxidative stress it was 0.09 mg Ni/m^3, and for DNA damage response it was 0.083 mg Ni/m^3 at 4 weeks timepoint. A similar inhalation study was also conducted for nickel sulfate, indicating that the highest concentration of 0.5 mg/m^3 (0.11 mg Ni/m^3) used in the NTP study might remain too low for tumorigenic responses.

Since the previous reports, a new *in vivo* inhalation study has been performed. Oller et al. (2023) performed 3 and 13 weeks study using nickel sulfate (0, 0.3, 0.11, 0.22, 0.44 mg Ni/m^3) and nickel subsulfide (0, 0.3, 0.11, 0.44 mg Ni/m^3). Since with nickel sulfate dose of 0.44 mg Ni/m^3 12/13 rats died or were euthanized due to pulmonary oedema, the highest dose was reduced to 0.22 mg Ni/m^3. Exposure to both compounds caused inflammation and cytotoxicity in a dose-dependent manner. Nickel sulfate was more potent in inducing bronchiolar epithelial apoptosis than nickel subsulfide. Nickel subsulfide, but not nickel sulfate, induced cell proliferation in epithelial and non-epithelial cells at the highest dose level, even though both substances caused inflammation. DNA damage, detected by the comet assay, was induced at the highest dose level (0.22 mg Ni/m^3) after 13 weeks of exposure to nickel sulfate. A dose-response was noticed after 3 and 13 weeks of exposure for nickel subsulfide. DNA damage with nickel subsulfide was detected at concentrations of 0.11 and 0.44 mg Ni/m^3. Oller et al. (2023) enriched type II epithelial cells for Comet analysis from the isolated lung cells but epithelial cell yield varied between the groups and remained very low. In addition, in the highest groups of both substances, nickel exposure induced pulmonary toxicity and inflammation, resulting in the presence of macrophages in the analyzed samples. Thus, DNA damage was detected in the lungs, but it cannot be specified to the epithelial cells, and the relevance for cancer risk might not be that evident. Hypermethylation was detected after 13 weeks of exposure to both substances. Oller et al. (2023) integrated the data from NTP studies (1996b, c) and from Efremenko et al. (2014, 2017) studies, as the nickel burden in the lungs remained at the similar level across the studies and a steady state was reached in 3-4 weeks. Thus, they concluded that NOAEC is also relevant for the 2-year study. The NOAEC for lung inflammation was determined to be 0.03 mg Ni/m^3 for lifetime exposure for nickel sulfate and subsulfide. This study also supported nonlinear threshold approach for carcinogenicity.

6.9.3 Human data

Limited data is available for human genotoxicity with inconsistent findings (ECHA, 2018; EFSA, 2015). Co-exposure to other known genotoxic substances such as chromium, makes the evaluation for nickel specific genotoxicity limited. Micronuclei in epithelial cells were studied from the buccal mucosa of nickel refinery workers and no differences were observed in the frequency of micronucleus epithelial cells when compared to controls (Kiilunen et al., 1997b). In addition, micronucleus frequencies and levels of nickel in air, urine, or blood were compared, but no relationship was found. The urine nickel concentration was 0.1-2 µmol/l (5.9-117.4 µg/L). In the lymphocytes of welders, DNA single strand breaks and sister chromatid exchange frequencies were increased but exposure was co-exposure with chromium (Werfel et al., 1998). No relationship between DNA single strand breaks, sister chromatic exchange, and blood nickel levels was detected. The blood nickel concentration was 4.6±11.4, and the chromium concentration in erythrocytes was 4.3±7.0. Biomonitoring data of nickel, chromium, cadmium, and lead were compared to oxidative DNA damage in a cross-sectional study in urban population in Germany (n=824, 1993-1994) (Merzenich et al., 2001). The urine nickel concentration (median 1.0 µg/L) was positively associated with oxidative DNA lesions, with an odds ratio of 2.15 for tertiles 1 versus 3, $P<0.05$. Welder's blood nickel and chromium concentrations were measured and compared to DNA damage detected by the comet assay and to micronucleus of buccal epithelial cells (Danadevi et al., 2004a). The welders´ nickel and chromium concentrations were higher than in the controls (132.39 µg/L versus 16.91 µg/L for nickel and 151.65 versus 17.86 µg/L for chromium). In addition, welders had more DNA damage and micronucleate cells than controls. Alabi et al. (2020) studied the blood level of Pb, Ni, Cd, and Cr and DNA damage in exfoliated buccal cells using the micronucleus test in teenage E-waste scavengers (age 13-19, n=95) at Alaba International electronic market, Lagos, Nigeria, and compared it to controls (n=104). E-waste scavengers had increased levels of micronuclei when compared to the controls, and there was a positive correlation between different metals blood concentration and the frequency of micronuclei. The nickel blood concentration (median 1.05 µg/L) was higher in the scavengers' group than in the controls (median 0.01 µg/L). Similarly, blood concentrations for other metals were elevated in the scavengers' group.

The Flemish Environment and Health Studies (FLEHS) data from several time periods were pooled (n=2283, age 14-18, year 1999-2018) and used to study exposure-effect associations (Koppen et al., 2020). Nickel was positively associated with the oxidative stress marker 8-oxodG in 2/4 studies but not in the pooled analysis.

Similarly, no association was found between nickel and the comet assay in the pooled analysis. The median nickel concentration in urine was 1.50 µg/g creatinine and in blood it was 1.07 µg/L.

Nickel urine concentration and micronucleus in buccal epithelial cells was determined from children placed with metal crowns (n=37) on day 1 and day 45 (Morán-Martínez et al., 2013). Nickel urine concentration increased from day 1 (2.12 ± 1.23 µg Ni/g creatinine) to day 45 (3.86 ± 2.96 µg Ni/g creatinine). Similarly, frequency of micronucleate cells increased from day 1 (4.67 ± 0.15) to day 45 (6.78 ± 0.167) statistically significantly. However, this effect cannot be determined to be nickel specific as it is alloyed with iron and chromium. ECHA (2018) has listed the effects of orthodontic appliances on micronucleus levels and the results have been inconsistent.

6.9.4 Summary

In summary, nickel is not clearly directly mutagenic, as only weak responses in mutagenicity tests have been observed (RAC, 2018). These changes are most likely due to pathways other than point mutations. *In vitro,* nickel compounds have, however, caused morphological transformation of mammalian cells and induced chromosomal aberrations and micronuclei, sister chromatid exchange, single-strand breaks, double-strand breaks, aneuploidy and spindle-inhibiting effects (ECHA, 2018; EFSA, 2020). It can be concluded that these effects are both aneugenic and clastogenic. *In vivo* data are not totally consistent, but they confirm the aneugenic and clastogenic *in vitro* findings of nickel. Limited human genotoxicity data do not indicate clear direct positive effects linked to nickel exposure (ECHA, 2018).

ECHA (2018) and EFSA (2020) concluded that the genotoxicity mode of action of nickel is likely to be indirect due to inflammation, production of reactive oxygen species, and inhibition of the DNA repair system. In addition, nickel has epigenetic effects. $Ni2^{+}$ must be delivered sufficiently to the nuclear sites of target cells to cause genotoxic effects. This is affected by the bioavailability and transportation of the different forms of nickel into the cell.

6.10 Carcinogenicity

IARC (2012) and ECHA (2018) have extensively reviewed carcinogenicity studies. Nickel compounds have been classified as known human carcinogens (Cat 1A), and nickel metal is a suspected carcinogen (cat 2). Epidemiological and animal data show that nickel compounds cause lung and nasal cavity cancer (ECHA, 2018; IARC, 2012). It is notable that the exposure route in epidemiological studies has been inhalation. In addition, exposure routes in animal studies, which have been positive for carcinogenicity, are inhalation, intramuscular and subcutaneous administration, and intraperitoneal, intrarenal, intratesticular, and intraocular injection. Data from nickel metal are more uncertain.

6.10.1 In vivo

In inhalation carcinogenicity studies on nickel oxide and nickel subsulfide, rats have been sensitive to malignant tumor induction but mice have not (NTP, 1996a, b). The main results of key animal studies are summarised in table IV.6. Nickel subsulfide is more potent in inducing malignant tumors than nickel oxide. Nickel oxide showed a threshold effect, as compound was not carcinogenic at a dose level of 0.5 mg Ni/m^3 (NTP, 1996a), but nickel subsulfide was carcinogenic already at a dose of 0.11 mg Ni/m^3 (NTP, 1996b). Nickel sulfate hexahydrate did not show carcinogenic effect in animal studies, but it has been carcinogenic in epidemiological studies (ECHA, 2018; NTP, 1996c). Nickel metal showed adrenal gland pheochromocytomas (benign and malignant) in males and combined adrenocortical adenomas/carcinomas in females in a dose-dependent manner. These tumors were detected at a dose level of 0.1 mg/m^3. The authors concluded that pheochromocytomas are secondary to respiratory toxicity and adrenocortical tumors fall within the historical control range.

For the general population, the oral route is the main exposure route. Thus, studies related to oral carcinogenicity are of higher interest when assessing the risk for the general population (EFSA, 2015). EFSA (2015; 2020) has summarized oral carcinogenicity studies and concluded that there is no support for the

carcinogenicity of soluble nickel via the oral route. Soluble nickel is considered to be the main concern as its bioavailability from the gastrointestinal tract is greater than insoluble or poorly soluble compounds. The main results of the most recent oral carcinogenicity study by Heim et al. (2007) are presented in able 6. Carcinogenicity of nickel sulfate hexahydrate was studied via oral gavage, but no exposure related tumors were detected. In another study, the carcinogenicity of nickel chloride was studied in mice that were pre-exposed to ultraviolet radiation (Uddin et al., 2007). Nickel chloride enhanced the carcinogenicity potential of the ultraviolet radiation to cause skin tumors, and it was concluded that nickel acts as a co-carcinogen.

6.10.2 Human

6.10.2.1 Lung cancer

ECHA (2018) has extensively reviewed epidemiological studies on nickel and cancer. Here, the main points are referred to and relevant literature for more recent studies is added. The pooled SYNERGY case-control study included 16,901 lung cancer cases and 20,965 control subjects from 14 different countries in Europe and Canada (Behrens et al., 2023). The Job exposure matrix (SYN-JEM and DOM-JEM) was used for exposure estimates, and cumulative lifetime exposure was calculated as the sum of the country/region-specific SYN-JEM estimates for each job and year. In men, cumulative exposure to nickel in the highest exposure group (>78.1µg/m^3-y) resulted in OR 1.29, 95 % CI being of 1.15-1.45 for lung cancer. The cumulative exposure of >30.9 to ≤78.1 µg/m^3-y. resulted in an OR of 1.20, with a 95% CI of 1.07-1.35 for lung cancer. In women, the effect was not as clear due to a smaller sample size, at the highest cumulative exposure group the OR for lung cancer was 1.29, with a 95 % CI of 0.60-2.86. This study supports the conclusion that nickel causes lung cancer.

Metallic Nickel

Epidemiologic studies do not show evidence for metallic nickel lung carcinogenesis.

A cohort from the Oak Ridge Gaseous diffusion plant in Tennessee (n=813, 1948-1953) included workers involved in the production of barrier material for use in the gaseous diffusion process of uranium enrichment (ICNCM, 1990). Their mortality was followed up until 1982. High purity nickel powder was used for the barrier material, and the median nickel concentration in the air samples was 0.13 mg Ni/m^3. 70% of the work was done in two areas where the median was 0.56 mg Ni/m^3 (90th percentile 1.8) and 0.70 mg Ni/m^3 (90th percentile 1.4 mg Ni/m^3). However, these values were considered to underestimate the exposure as they were based on data from 1948-1963 when the working conditions had already improved. The authors estimated that the average airborne metallic nickel concentration was below 1 mg Ni/m^3. No excess risk for lung cancer was detected (SMR 0.54, 95% CI 0.25-1.0).

A cohort from a hydrometallurgical nickel refinery in Fort Saskatchewan, Alberta, Canada (n=1694, 1954-1978) included workers who were exposed to metallic nickel and nickel concentrates (Egedahl et al., 2001). Their mortality was followed up until 1995. Exposure to metallic nickel was 2 mg Ni/m^3 (range 0.7-3 mg Ni/m^3) and 4 mg Ni/m^3 (range 0.3-49 mg Ni/m^3) at two high volume areas. No excess risk for lung cancer was detected (SMR 0.67, 95% CI 0.24-1.5), but the healthy worker effect may have caused bias in the results, as overall mortality was also lower than in the general population.

Nickel alloy workers (n=31165) from 13 US plants were included in the cohort (Arena et al., 1998). Since there were limited measurement data on exposures, the study used the time of employment and exposure estimates from specific work tasks as surrogate measures of cumulative exposure. This data was mainly based on one plant in the late 1970s. Powder metallurgy had average airborne nickel concentrations of 1.5 mg/m^3, the grinding operation had 0.3 mg/m^3, and the hot working areas had 0.1 mg/m^3. In other areas, mean nickel concentrations were lower. However, in another study that reconstructed historical exposure in process areas outside of powder metallurgy, the range was approximately 0.3-1.8 mg Ni/m^3, with an overall average of 0.65 mg Ni/m^3 (1.5 mg/m^3 as inhalable) (Sivulka and Seilkop, 2009). A slightly increased risk of lung cancer was detected in the workers of nickel alloy production with an SMR of 1.13 when compared to US national rates, but no increase was detected when compared to local rates (Arena et al., 1998)

The Hereford, UK cohort included workers from nickel alloy manufacturing (1952-1992) (Sorahan, 2004). In personal air samples, mean levels ranged between different work tasks from 0.04 to 0.84 mg Ni/m^3 during 1975-1980 and from 0.29 to 0.45 mg Ni/m^3 during 1997-2001. No increased risk of lung cancer was detected (SMR 0.87; 95% CI 0.67 – 1.1). The healthy worker effect was observed as total mortality and lung cancer mortality rates decreased when compared to the male mortality rates of England and Wales.

At the Kristiansand electrolytic refinery, risks related to different nickel compounds were studied based on their presence in the different phases of production (Grimsrud et al., 2002). Metallic nickel had an increased lung cancer risk (OR 2.4, 95% CI 1.1-5.3) in the highest exposure group (mean 2.3 mg/m^3 x years), but after adjusting for water-soluble nickel compounds, the risk did not remain significant (OR 0.9, 95% CI 0.3-2.4). The results were adjusted for cigarette smoking.

In addition, in Clydach refinery, workers lung cancer risk related to different species were studied. Initial results, studied with a linear model suggested increased risk related to metallic nickel. However, the data was later re-calculated using a cross-validation test, which was performed including only workers employed after 1930, and the predicted cancer cases from the model were then compared to the observed cancer cases. It was concluded that the data overestimated metallic nickel risk (and possible soluble nickel) and underestimated those for sulfides or oxides (Easton et al., 1992; ECHA, 2018).

Water soluble nickel compounds

The key studies for soluble nickel compounds are from Kristiansand, Harjavalta, and Clydach nickel refineries. These studies indicate dose-related lung carcinogenicity for water-soluble nickel compounds.

Kristiansand is an electrolytic nickel refinery in Norway that has been operating since 1910 (Grimsrud et al., 2000). Nickel sulfate was the main exposure source until 1978, but after renewing the process, nickel chloride became the main source in the nickel electrolysis. After 1978, nickel sulfate was found in the roasting, smelting, and some other departments. In the electrolysis department and electrolyte purification, nickel sulfate, and/or nickel chloride are the main nickel species, with a proportion of 80-85% (by weight). For all workers, the average concentration of nickel in the breathing zone was < 0.7 mg/m^3 after 1978. Before 1970, exposure levels for smelter and roaster workers were 2-6 mg/m^3, and for workers in the electrolysis department and electrolyte purification, they were 0.15 – 1.2 mg/m^3. Andersen et al. (1996) found an increased lung cancer risk (standardized incidence ratio (SIR) 3.0, 95% CI 2.6-3.4) during the follow-up period (1953-1993) at the Kristiansand nickel refinery. A dose-response was detected for soluble nickel compounds and for nickel oxide, although the linear trend was not as strong as for soluble nickel compounds. Smoking was suggested to cause a multiplicative effect with nickel for lung cancer. Grimsrud et al. (2002) found a dose-response for soluble nickel compounds when adjusted for smoking. A constant risk (odds ratio) for water-soluble dose-response of 1.5 was observed regardless of exposure level (i.e. any exposure vs unexposed) and a unit increase of risk (odds ratio) of 1.7 per unit in the natural log-transformed exposure given originally in (mg/m^3) x years. The dose-response for water-soluble nickel compounds was confirmed in another Grimsrud et al. (2003) study. The lung cancer risk of water-soluble compounds persisted even after adjusting for other known carcinogens in the refinery (Grimsrud et al., 2005). Grimsrud et al. (2002) data has been criticized for underestimating the exposure at low doses (Goodman et al., 2011). Goodman et al. (2011) concluded that lung cancer risk is strongly associated with working in electrolysis (with no roasting, smelting, or calcining exposure) in Kristiansand (SMR = 385, 95% CI: 259–549 based on ICNCM (1990); SIR = 510, 95% CI: 320–770 based on Grimsrud et al. (2002)).

The Harjavalta cohort is from a smelter and electrolytic refinery where workers are exposed to nickel matte (a mixture of nickel, nickel sulfides, and subsulfides), nickel sulfides and nickel subsulfides (Anttila et al., 1998). Before 1973, exposure was a combination of soluble and insoluble nickel compounds due to leaching and grinding operations that were in the same place. Personal nickel exposure levels for smelters were 0.02-0.2 mg/m^3 but one measurement was 0.7 mg/m^3. In electrolysis, personal nickel exposures were estimated to be 0.25 mg Ni/m^3. An increased lung cancer incidence was observed in the electrolytic refinery with a SIR of 2.6, 95% CI 1.0-5.7. The risk increased after adjusting for a 20-year latency period. For smelter workers, the risk was a SIR of 1.4, 95% CI 0.8-1.6. Pavela et al. (2017) conducted a study in Harjavalta where they

followed up nickel exposed workers from 1967 to 2011. At the nickel refinery site, the lung cancer risk was a SIR of 2.61, 95% CI 1.10-3.36%.

The Clydach cohort is from a nickel refinery that opened in 1910 and operated until the end of 1969, with follow-up until 1984 (n=2521) (ICNCM, 1990). It uses the Mond nickel carbonyl process, and exposure to different species varied between departments. Water-soluble nickel was the main nickel compound in the hydrometallurgy department, ranging from 30-40% up to 100% in drying and bagging. Working in the hydrometallurgy department (>5 years) and less than one year in the other high-risk departments, raised lung cancer mortality 3-fold (SMR 333, P=0.019).

Poorly soluble nickel compounds

Sulfidic and oxidic nickel are present in roasting, smelting, and calcining processes (ICNCM, 1990). Sulfidic nickel is calcined to oxides in the nickel producing plants (ECHA, 2018). Thus, it is difficult to distinguish between these compounds. In addition, most of the processes include other nickel compounds, making the specific nickel compound analysis challenging.

In the Kristiansand cohort, exposure to nickel oxide showed an increased risk of 1.5 (95% CI 1.0-2.2) and a linear trend for dose-response (p=0.05) when adjusted for smoking, age, and soluble nickel. However, Grimsrud et al. (2002) did not find this increased risk after they improved the exposure estimate.

The ICNCM report (1990) concluded that in the Clydach refinery an increased risk (SMR 638, p = 0.004) for lung cancer was found for high sulfidic nickel exposure (≥ 15 (mg Ni/m^3) x years) with low exposure to oxidic and soluble nickel. For nickel oxide, increased risk for lung cancer reached statistical significance only with co-exposure to soluble nickel compounds. In another study from the Clydach cohort, the effect of poorly soluble nickel compound has been studied, and no increased risk was found but it was concluded that the study underestimated the risk of nickel sulfides and oxides (Easton et al., 1992; ECHA, 2018).

The Ontario cohort, which included workers from the Copper cliff sintering plant and Port Colborne leaching, calcining, and sintering departments, and the Coniston sintering department showed increased lung cancer risk (SMR 261, 95% CI 220-306). The nickel concentrations at Copper Cliff were 25-60 mg Ni/m^3 oxidic nickel, 15-35 mg Ni/m^3 sulfidic nickel, and 4 mg Ni/m^3 soluble nickel. Port Colborne had similar exposure levels, but Coniston had lower exposure levels. Sub-cohorts of Ontario nickel workers, Copper cliff sintering plant (worked ≥5 years, SMR 789, p<0.001) and Port Colborne leaching, calcining, and sintering departments (worked ≥5 years, SMR 366, p<0.001) showed an even higher increased risk for lung cancer.

In the other two cohorts, Hanna mining cohort (Oregon) and Societé le Nickel cohort (New Caledonia), no increased risk for lung cancer was detected but the exposure levels remained very low when compared to other cohorts (<1 mg Ni/m^3) (ICNCM, 1990).

6.10.2.2 Nasal cancer

At the Kristiansand nickel refinery, the risk of nasal cancer was increased (SIR 18.0, 95% CI 12-25) (Andersen et al., 1996). The total cumulative exposure to nickel oxide with soluble nickel exposure <1 mg Ni/m^3 was SIR 13.7, 95 % CI 7.5-23.0. When the soluble nickel air concentration was ≥15 mg Ni/m^3, the risk increased enormously (SIR 81.7, 95% CI 45-135). In the Harjavalta nickel refinery workers, nasal cancer risk was clearly increased (SIR 41.1, 95% CI 4.97±148) after a 20-year latency period (Anttila et al., 1998). The main exposure source in the refinery was nickel sulfate. Increased nasal cancer risk (SIR 26.68, 95% CI 5.50–77.97) was also seen in a more recent study from Harjavalta (Pavela et al., 2017). According to the ICNCM report (1990), the nasal cancer risk was increased at the Clydach nickel refinery. The evidence was clearest for sulfidic nickel and soluble nickel, whereas metallic nickel did not increase the risk. Easton et al. (1992) found that only soluble nickel significantly increased the risk of nasal cancer. Authors concluded based on lung cancer risk of metallic nickel that the model underestimates the risk of sulfides and oxides and overestimates the risk of metallic and possibly soluble nickel (ECHA, 2018). In the Ontario cohort, an increased nasal cancer risk was evident (SMR 5073, 95 % CI 3282-7489) after 15 years of employment. Seilkop et al. (2017) confirmed the increased nasal cancer risk in the Copper Cliff sinter facility and Port Colborne leaching,

calcining, and sintering facilities, which are subcohorts of the Ontario cohort. At the Oak Ridge Gaseous diffusion plant no increased risk was detected for metallic nickel (ICNCM, 1990). In addition, increased nasal cancer risk was also detected in the Swedish Ni-Cd battery factory (Järup et al., 1998).

6.10.2.3 Other cancers

No convincing data has been found for any other cancer types than lung and nasal cancer (ECHA, 2018; IARC, 2012). An increased risk for stomach cancer (SIR 4.98, 95% CI 1.62±11.6) was found after 20 year latency period in the nickel refinery workers who were mainly exposed to nickel sulfate (Anttila et al., 1998). However, in the latest follow-up, no significant increase was found (SIR 2.45, 95% CI 0.79–5.70) (Pavela et al., 2017). Lightfoot et al. (2017) did not find an increased risk for stomach cancer in the Ontario cohort. An increased buccal cavity cancer risk (SMR 211, 95 % CI 211 109-369) was found in the Ontario cohort when compared to Ontario mortality rates (ICNCM, 1990). However, in the Lightfoot et al. (2017) study, there was no significantly increased risk in lip and oral cavity cancer mortality or incidence. Arena et al. (1998) found an increased risk of colon cancer in non-white males (RR 1.92, 95% CI: 1.28–2.76) and a 2-fold increase in the risk of kidney cancer in white males working in melting. However, the length of employment or time since first employment did not affect the risk. Lightfoot et al. (2017) did not find an increased risk for colon or kidney cancer.

6.10.2.4 General population

The effects of air pollution on lung cancer incidence were studied in the ELAPSE study. The PM2.5 pollutant nickel (1 ng/m^3) increased the hazard ratio for lung cancer incidence (1.09 HR, 95% CI 1.02-1.15), but when adjusted for PM2.5, the increase did not remain significant (1.05 HR, 95% CI 0.99-1.12) (Hvidtfeldt et al., 2021).

In the air pollution ELAPSE study, the effects of air pollutants on liver cancer incidence were studied (So et al., 2021). Nickel (0.8 ng/m^3) was studied among PM2.5 pollutants, and single pollutant analysis, using supervised linear regression exposure model, showed hazard ratio of 1.19 (95% CI 1.05 to 1.35). However, when adjusted for other PMs in the random forest analysis, no significant effect (1.13 HR, 95% CI 0.80 to 1.60) was detected.

6.10.3 Summary

In *in vivo* studies, nickel subsulfide and nickel oxide have been carcinogenic in rats, but nickel sulfate has remained negative when exposed via inhalation (ECHA, 2018). In contrast to *in vivo* studies, epidemiological studies indicate a dose-dependent lung and nasal cancer from water-soluble nickel compounds. Epidemiological data also points that insoluble nickel oxide and subsulfide induce lung and nasal cancer. However, as noted by ECHA (2018), it is challenging to identify the role of exact nickel species in epidemiological data, which may explain some inconsistencies between the studies on whether soluble or insoluble compounds are considered as the main carcinogen. Oller et al. (2014) stated that no cohort shows an increased lung cancer risk if the concentration of sulfidic nickel remains below 0.02 mg Ni/m^3, soluble nickel below 0.1 mg Ni/m^3 and oxidic nickel below 2 mg Ni/m^3. Nickel metal has been negative in relevant animal carcinogenic studies, but epidemiological studies cannot exclude the potential for carcinogenicity (ECHA, 2018). Differences in the carcinogenic potential are probably due to differences in the cellular and nucleus uptake of the nickel compounds.

Transportation into cells might be through ion channels (water-soluble nickel compounds) or phagocytosis (poorly soluble). Based on *in vitro* studies, the cellular uptake is lowest for water-soluble nickel, < metallic nickel < amorphous nickel sulfide < nickel oxide < nickel-copper oxides < crystalline nickel sulfide < crystalline nickel subsulfide.

Although nickel subsulfide and nickel sulfate had similar severity of inflammation in the lungs in the chronic toxicity studies there were differences in the carcinogenicity. Nickel subsulfide was carcinogenic whereas

nickel sulfate did not cause benign or malignant tumors (ECHA, 2018). Carcinogenicity differences has been explained by retained dose, clearance, target cell uptake, and intracellular uptake.

Nickel sulfate has a low retained dose and very rapid clearance. Although it has high extracellular dissolution, it has low intracellular dissolution leading to very low release of nickel ions nearby nucleus. Thus, no carcinogenicity has been detected in animals exposed to nickel sulfate (Buxton et al., 2019). Whereas nickel subsulfide has low retained dose, rapid clearance, and medium extracellular dissolution. However, it has high intracellular dissolution leading to the high release of nickel ions nearby nucleus and sufficient bioavailability into the nucleus. The bioavailability into the nucleus is sufficient enough to cause carcinogenicity in rats (Buxton et al., 2019). Good intracellular dissolution is probably also the reason why nickel subsulfide has caused higher toxicity than nickel oxide (ECHA, 2018). Metallic nickel has low endocytotic uptake and slow intracellular dissolution, leading to a low amount of nickel ions at nuclear sites of respiratory target cells.

RAC (2018) concluded that nickel compounds are genotoxic carcinogens with a mode-of-action based threshold. Critical effects in the lung are inflammation and cytotoxicity, leading to misreplication and secondary genotoxicity (RAC, 2018).

EFSA (2020) stated in their report that it is unlikely that nickel causes cancer in humans after oral exposure based on animal studies being negative for cancer after oral exposure to soluble nickel, and the mode of action.

7 Overview of existing toxicity reference values for the general population

Table IV.9 lists toxicity reference values for the general population. EFSA updated the evaluation for TDI in 2020. In this update, they used the EFSA BMD guidance (2017) to calculate $BMDL_{10}$. The same SLI (2000a, b) studies have been used to determine the point of departure in all of the TRV assessments. SLI performed a one-generation reproduction range-finding study and a two-generation reproductive toxicity study on Sprague-Dawley rats. The NOAEL was determined to be 2.2 mg/kg bw/day and most critical effects of nickel were post-implantation loss in the offspring and an increase in dead pups.

In addition, EFSA and WHO considered acute toxicity of nickel oral exposure based on SCD. WHO stated that "*For acute toxicity, the SCD elicited in nickel-sensitive humans after exposure to nickel through water is the most sensitive and critical effect".* However, EFSA did not set a guidance value for acute effects due to insufficient data instead they used margin of exposure (MOE) approach (EFSA, 2020). EFSA stated that MOE raised a health concern for nickel-sensitized individuals. WHO used MOE approach from EFSA to evaluate acute exposure scenario (a bolus exposure) that was considered to be of low health concern (WHO, 2021). This bolus exposure differs from normal drinking water exposure scenario that has intermittent nature of a normal drinking-water exposure scenario. In addition to the values in the table IV.9, ATSDR has evaluated minimal risk level for nickel oral exposure but did not set minimal risk level due to insufficient data for acute, intermediate, or chronic exposure (ATSDR, 2024).

Table IV.11: Toxicity reference values for nickel

Agency	Key study	Endpoint	Point of departure (mg/kg bw/d)	Assessment factors	TRV
ATSDR (2024) Intermediate	(Oller et al., 2023)	Inflammation in respiratory tract	BMDL of 0.0014 mg Ni/m^3 ($BMDL_{HEC}$ of 0.0000982 mg Ni/m^3)	30	$3x10^{-6}$ mg Ni/m^3 =3 ng/m^3
ATSDR (2024) acute	(Efremenko et al., 2017)	Bronchiole epithelial degeneration/hyperplasia	LOAEL of 0.2244 mg Ni/m^3 ($LOAEL_{HEC}$ of 0.0403 mg Ni/m^3)	300	$1x10^{-4}$ mg Ni/m^3 =400 ng/m^3
WHO (2021)	(SLI, 2000a, b)	Reproductive and developmental toxicity	$BMDL_{10}$ 1.3	allocation factor 20%	Drinking water guidance value 70 µg/L.
(EFSA, 2020)	Combination of (SLI, 2000a, b)	Reproductive and developmental toxicity	$BMDL_{10}$ 1.3	100	TDI 13 µg/kg bw/d
(EFSA, 2015)	Combination of (SLI, 2000a, b)	Reproductive and developmental toxicity	$BMDL_{10}$ 0.28	100	TDI 2.8 µg/kg bw/d

Agency	Key study	Endpoint	Point of departure (mg/kg bw/d)	Assessment factors	TRV
Danish EPA (2013)	(SLI, 2000b)	Developmental toxicity	1.1	200	TDI 5.5 µg/kg bw/d
				allocation factor 10%	From TDI, quality criterion for drinking water for soluble inorganic nickel salts was derived: 20 µg Ni/L.
WHO (2000)	Studies in occupationally exposed human populations.	Carcinogenicity	Incremental risk of 3.8 × 10^{-4} can be given for a concentration of nickel in air of 1 µg/m^3		lifetime risk of 1:10 000, 1:100 000 and 1: 1 000 000 are about 250, 25 and 2.5 ng/m^3, respectively.
US-EPA 1991	Nickel refinery human data	Carcinogenicity	2.4x10^{-4} µg/m^3	Multiplication factor of 2 to account for the roughly 50% nickel subsulfide composition	Inhalation unit risk for general population for nickel subsulfide 4.8x10^{-4} µg/m^3
CEPN (1997)	Studies in occupationally exposed human populations.	Lung cancer	2.5 x 10^{-4} unit risk for continuous lifetime exposure to 1 µg Ni/m^3 linear non-threshold approach	Adjustments made based on animal data	For exposure to 1 µg Ni/m^3 cancer risk estimate for lifetime for nickel oxide: 4.0 x 10^{-5} unit risk and for Nickel subsulfide: 3.0 x 10^{-4} unit risk
EU council directive 98/83/EC					20 µg/L

8 Derivation of HBM-GVs for nickel with regard to the general population

Apel et al. (2020) established three possible ways to set an HBM-GV: HBM-GV derivation from human data based on a relationship between internal concentrations and health effects, HBM-GV derivation based on a defined external toxicity reference value or on a defined occupational exposure limit, and derivation based on a critical effect observed in experimental animal studies.

There are limited data to derive HBM-GV for nickel based on human internal concentration of exposure biomarker and health effects. In addition, although skin sensitization is studied thoroughly, nickel is not well absorbed through the skin. Thus, no correlation between the levels causing skin sensitization and urine nickel levels can be made.

However, suitable toxicity reference values based on reproductive toxicity are available for the derivation of an HBM-GV. Thus, this approach can be used to derive nickel HBM-GV for the general population. Acute toxicity based systemic contact dermatitis (SCD) was also considered but there was insufficient data to use this endpoint as a basis for HBM-GV derivation.

9 Choice of the TRV for the derivation of HBM-GV for general population

Most of the TRV values are based on the same reproductive studies as key studies (SLI, 2000a, b). WHO (2000) for air quality guidelines, US-EPA, and CEPN have chosen carcinogenicity as a critical effect. ATSDR determined respiratory effects as the most critical effect. WHO calculated an incremental risk of 3.8×10^{-4} for a concentration of nickel in the air of 1 µg/m³ using linear-dose response approach based on industrial populations. It should be noted that WHO cancer risk estimate calculations were based on total dust measurement, not on inhalable dust (ECHA, 2018). The correction for inhalable dust was estimated to lower the risk by approximately 50%. In addition, the current view is that nickel compounds are genotoxic carcinogens with a mode-of-action based threshold (RAC, 2018). Thus, WHO estimate is rather conservative.

Ambient air concentrations at non-industrial sites have been around or below 3 ng/m³, and at industrial sites, they have been up to tens to hundreds ng/m³ (EFSA, 2020). In the general population, the main exposure route is the oral route, accounting for around 99% of the exposure, while the inhalation route represents only a minor fraction. It can be estimated whether the cancer risk from air nickel exposure is relevant when compared to oral exposure. The WHO (2000) calculated lifetime cancer risk 1:1,000,000 at a nickel concentration of 2.5 ng/m³. This can be converted to kg bw/day, resulting in 0.71 ng/kg bw/day (Formula 1). In the recent evaluation of ATSDR, minimal risk level for intermediate inhalation exposure of 3 ng/m³ was determined in general population based on inflammation in respiratory system. This can be converted to 0.86 ng/kg bw/day. For acute effects minimal risk level was determined to be 400 ng/m³ corresponding to 0.11 µg/kg bw/day.

$$oral\ exposure = \frac{air\ concentration * Inhalation\ volume}{body\ mass}$$

$$oral\ exposure = \frac{2.5\frac{ng}{m^3} * 20\frac{m^3}{day}}{70\ kg} = 0.71\frac{ng}{kg}/day$$

Formula IV.1: Inhalation exposure converted into oral exposure. Standard values for dose calculations: inhalation volume for the general population is 20 m³/day and body mass is 70 kg (ECHA, 2012).

In adults, in European countries, the mean dietary exposure in the total population has a maximum range between the lower bound and upper bound of 3.65-4.19 µg/kg bw/day, and a mean range between the lower bound and upper bound of 2.90-3.41 µg/kg bw/day (EFSA, 2020). For children/toddlers, these values can be three times higher. Ambient air exposure levels in non-industrial sites and industrial sites are minimal when compared to dietary exposure levels. Thus, it can be concluded that the cancer risk or respiratory toxicity at relevant urban air levels through the air is minimal when compared to food exposure. Carcinogenicity or respiratory toxicity has been detected only after inhalation exposure; thus, it can be concluded that carcinogenicity or respiratory toxicity are not the most critical effect for the general population. Thus, other TRV values that use SLI (2000a, b) studies can be considered as the starting point for the derivation of the HBM-GV.

The BMD approach is considered to be the state-of-the-art method for choosing the point of departure. EFSA has used this approach to set the TDI value for nickel. In 2020, EFSA updated their assessment for TDI based on new occurrence/exposure data, updated guidance to perform BMD, and new scientific information. Additionally, the EFSA TDI value considers exposure to both food and drinking water in contrast to TRV values that are made only for drinking water, as derived by Danish EPA (2013), WHO (2021), or set by EU Council. Thus, the EFSA (2020) TDI value of 13 µg/kg bw/day can be chosen as the starting point for the derivation of HBM-GV for the general population.

10 Derivation of HBM-GV for general population

10.1 Urinary mass balance approach

Based on the mass balance equation, HBM-GVs for the general population can be derived. The general population is mainly exposed to nickel via food and water (EFSA, 2020). Nickel is primarily measured as total nickel in food, and information on speciation is limited (EFSA, 2020). However, a general assumption has been made that nickel occurs in food in the form of complex bound organic nickel. This form has different physico-chemical and perhaps also biological properties than inorganic nickel (EFSA, 2020).

There are only a few toxicokinetic studies on nickel after oral exposure. Excretion of nickel for 3 days has been between 0.7-27%. Excretion has high variability and is affected, for example, by fasting status and solubility of nickel. Calculations for HBM-GV can be made using the lowest and highest excretion values. The lowest value stands for the situation where nickel is administered with food and highest value when nickel has been administered during fasting. Sunderman et al. (1989) studied nickel sulfate excretion at 3 different dose levels: 12 µg Ni/kg bw (n=4), 18 µg Ni/kg bw (n=4), 50 µg Ni/kg bw (n=1). The dose did not affect the excretion. Median excretion was 0.7% when nickel was administered with food and 27 % without food. In the study conducted by Nielsen et al. (1999), nickel supplemented water (the form of nickel used was not determined) was used with a dose of 12 µg Ni/kg bw (n=8). The median excretion value was 2.51% when administered with food and 25.81% without food. Both studies were well conducted. The average of these two studies is 1.6% and 26.4%. Calculations were done using the excretion value for 3 days as no numerical data for the 24-hour excretion was available. From the Sunderman et al. (1989) study, it can be estimated that the cumulative excretion value is lower at the 24-hour time point. In the fasting status, cumulative excretion might be even 10% less at the 24-hour than the 72-hour time point. However, when nickel sulfate was administered with food, the effect seems to be less relevant as cumulative excretion is low in both time points. Fasting status causes the greater uncertainty than the extrapolation of the different timepoints.

$$HBM - GVGenPop = \frac{TRV * Fue(substance)}{Daily\ urinary\ flow\ rate\ adjusted\ to\ the\ bw}$$

Formula IV.2: Different HBM-GV options are calculated by the urinary mass balance equation. TRV=13 µg/kg bw/day (EFSA, 2020), urinary flow rate: 0.03 L/kg bw/day for children and men; 0.035 L/kg bw/day for women (German HBM Commission, 2025, submitted), Fue, when administered with food is 0.007 (Sunderman et al., 1989) or 0.0251 (Nielsen et al., 1999) and when administered without food, it is 0.27 (Sunderman et al., 1989) or 0.2581 (Nielsen et al., 1999).

Table IV.12: Different HBM-GVs for nickel calculated using Fue based on non-fasting and fasting conditions

Parameter source	HBM-GV on non-fasting conditions (µg/L)	HBM-GV on fasting conditions (µg/L)
Sunderman et al. (1989)	3.03 for children and men; 2.6 for women	117 for children and men; 100.29 for women
Nielsen et al. (1999)	10.88 for children and men; 9.32 for women	111.84 for children and men; 95.87 for women

By using the mass balance equation (Formula 2) (Apel et al., 2020), HBM-GVs for the general population can vary between 2.6 µg/L and 117 µg/L depending on age, sex and fasting status (Table IV.10). As the deviation between calculated HBM-GVs is high, the uncertainty is also high. In addition, it should be noted that calculations are based on toxicokinetic studies that used nickel sulfate (Sundermann et al.,1989) or nickel

supplemented water (the form of used nickel was not determined) (Nielsen et al., 1999). As it is estimated that nickel is mainly in a complex bound organic form in food, the absorption and bioavailability may be different than for nickel sulfate and probably won´t be absorbed as well as pure nickel sulfate (EFSA, 2020). This causes uncertainties in the HBM-GV estimations.

10.2 PBPK modelling

Predictions of HBM-GV for the general population were made using the PBPK model of Dede et al. (2018). The alimentary absorption of Ni from the dietary (baseline) Kf used by Dede et al. (Dede et al., 2018) was not considered in the developed PBPK model, as the TRV of 13 (µg/kg bw/day) is considered to be the total exposure, as shown in figure 1.

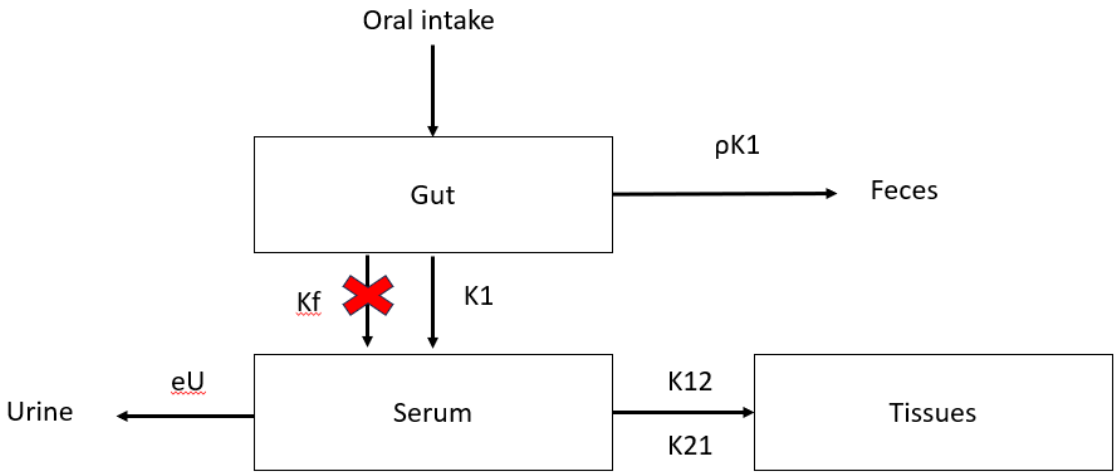

Figure IV.1: PBPK model modified from Dede et al. (2018). K1, K12, K21, Kf = transfer rates between compartments. eU=rate constant of urine elimination. Agut= Absorbed fraction of Ni dose. ρK1= rate constant for faecal excretion, ρ= 1-Agut/Agut

Two oral exposure conditions were simulated: once in a fasting state (Fraction absorbed by the gut (Fue) = 0.27, according to Sunderman et al. 1989 / Fraction absorbed by the gut (Fue) = 0.2581, according to Nielsen et al. 1999) and once in a non-fasting state (Fraction absorbed by the gut (Fue) = 0.007, according to Sunderman et al. 1989 / Fraction absorbed by the gut (Fue) = 0.0251, according to Nielsen et al. 1999). The oral exposure concentration was TRV=13 µg/kg bw/day, which means that the individual ingested 13 (µg /kg bw/day) of Nickel (Ni^{2+}). The body weight (bw) was assumed to be 70 kg.

Table IV.13: Different HBM-GVs for nickel when calculated using PBPK modelling on non-fasting and fasting conditions

Parameter source	Exposure conditions	Fraction absorbed by gut (Agut) [-]	Daily urinary flow rate (adults) [L/kg bw/day][1]	HBM-GV predictions (adults) [µg/L]
Sunderman et al. (1989)	Non-fasting	0.007	0.02	0.69
	Fasting	0.27	0.02	165.16
Nielsen et al. (1999)	Non-fasting	0.025	0.02	6.2
	Fasting	0.26	0.02	156.62

[1] *Daily urinary flow rate is based on (Apel et al., 2020). For organisational reasons, it was not possible to update the calculations with the revised daily urinary flow rates.*

Similar to the urinary mass balance approach, high variability in the predictions was detected in PBPK modelling. The predicted HBM-GVs for adults varied between 0.69 µg/L and 165 µg/L, based on the old standard value for the body weight-related 24h-urine volume and depending on fasting status and used fraction absorbed by gut. (For comparison: When using the urinary mass balance approach and the old default value for the 24h-urine volume for adults, the range was between 4.55 µg/L and 176 µg/L.) The model is highly dependent on the absorption rate from the gut into the bloodstream. In addition, the PBPK model is based on parameters from studies that used nickel sulfate. Thus, the HBM-GVs derived from the PBPK modelling has similar uncertainty as the urinary fraction approach due to possible differences in the absorption and bioavailability of the different form of nickel in food than those used in the studies (EFSA, 2020). All in all, it can be concluded that high uncertainty remains in both the urinary mass balance and PBPK modelling approaches, also due to the fact that they are based on different form of nickel that is estimated to be in the food.

10.3 Comparison of the possible HBM-GVs for the general population

For the general population, the main exposure source is food and water, thus, non-fasting condition can be considered the most relevant for HBM-GV for the general population. However, exposure through drinking can also occur on a fasting status. The most conservative HBM-GVs were estimated from calculations or predictions made for non-fasting status. For the general population, the critical effect is the reproductive toxicity, which concerns both women and men. It has been observed that cumulative excretion of nickel is lower in women than in men (Nielsen et al., 1999). The Nielsen et al. (1999) kinetic study included only men, but the Sunderman et al. (1989) study included both men and women.

Otherwise, both studies were conducted well and in both studies the participant number was less than 10. Moreover, the differences in the form of nickel in the food and the parameter source for HBM-GV estimation cause uncertainties. Toxicokinetic studies used nickel sulfate and nickel supplemented water, but nickel is mainly in a complex bound organic form in food. This causes uncertainties as there might be a deviation between the nickel uptake in food and nickel sulfate. Thus, nickel sulfate may not be the most suitable surrogate for nickel in food. All in all, both studies have been conducted similarly, and it cannot be determined which estimation is most reliable. However, when considering the uncertainty due to high deviation of excretion values and effect of fasting status, the lowest predicted HBM-GV is considered too conservative and is excluded.

Due to the uncertainties related to the high deviation of excretion caused by fasting status, and differences in the form of nickel causing uncertainty in the uptake and clearance of nickel, it was abstained to derive HBM-GVs as the spectrum of possible HBM-GVs is so wide that it makes it difficult to use it for EU-wide policy

making. **However, an orientation HBM-GV range is proposed ranging from 3 to 11 µg/L which refers to the non-fasting status only.** This orientation HBM-GV range can be used for local decision making.

A range is specified because a defined value cannot be derived due to too many influencing variables. This indication is not to be confused with a provisional HBM-GV, which has been derived with good reason but has not yet been harmonized within PARC. When it is known that a nickel urine sample has been taken in a non-fasting status, an initial rough assessment for possible increased risk for health concern could be made. Further investigations with additional determination of relevant influencing variables would be necessary in order to be able to make more detailed statements.

When the HBM measurement is below the lower limit value of the orientation HBM-GV range it can be concluded that health effects are unlikely. If measured HBM value is between orientation HBM-GV range, health concern cannot be excluded. Exceeding the orientation HBM-GV range suggests a potential human health concern. Orientation HBM-GV range can be used to identify potential hot spot areas and to monitor the effectiveness of the mitigation measures to reduce exposure at those hot spot areas.

11 Level of confidence attributed to the derived orientation HBM-GV range for the general population

When deriving HBM-GVs, the level of confidence in the derived HBM-GV should be estimated as high, medium, or low (Apel et al., 2020; OECD, 2022). The level of confidence proposed here is based on expert judgement about the reliability of the data and the calculation method used in the HBM-GV derivation. Here, it was abstained to derive HBM-GV due to high uncertainties instead orientation HBM-GV range was proposed.

The level of confidence for nickel orientation HBM-GV range for the general population can be estimated to be low as data from the relationship between internal concentrations and health effects is limited and there is considerable variability in the excreted fraction. In addition, little is known about the form of nickel in food as well as its toxicokinetics. The estimation of orientation HBM-GV range is extrapolated from nickel sulfate which may have different absorption and bioavailability than complex bound organic nickel. Complex bound organic nickel is the form of nickel that has been estimated to be in food.

12 Overview of existing occupational exposure values

Table IV.14: Existing occupational exposure values. Table combined from the data of (ECHA, 2018)

Agency	Key study	Endpoint	Point of departure (mg Ni/ m³)	Assessment factors	OEL (mg/m³)
RAC (2018)	(NTP, 1996a, b, c)	Inflammatory effects, lung fibrosis and lung cancer	Nickel sulfate: NOAEC 0.03 Nickel subsulfide: LOAEC 0.11 Nickel oxide: LOAEC 0.5	For nickel subsulfide and nickel oxide. AF 3 LOAEC→NOAEC→ calculated HEC (human equivalent concentration) 0.03 For nickel oxide, subsulfide and sulfate →no need of AF for toxicokinetics, AF1 for toxicodynamics, intraspecies variability AF 3, → AF 2 for severity of the endpoint	0.005 for respirable dust
RAC (2018)	Kristiansand cohort (Grimsrud et al., 2002; Grimsrud et al., 2003)	Nasal cancer	0.04	AF 3 LOAEC to NOAEC, → correction factor 2 for historical changes	0.03 for inhalable dust
SCOEL (2011)	(NTP, 1996c)	Inflammatory effects in lung that should also protect against carcinogenicity. Indirect genotoxic MOA is considered	0.03	HEC lead to 0.016 mg/m³ and then AF 3 was applied	0.005 for the respirable fraction
SCOEL (2011)	(Grimsrud et al., 2002)	Nasal cancer	0.04		0.01 for the inhalable fraction (Do not apply for metallic nickel)
NiPERA 2017	Integrated high number of human data	Lung cancer		HEC was applied	0.01 respirable 0.05 inhalable DNEL
AGS 2017	(NTP, 1996c)	Lung inflammation	0.027 NOAEC	HEC→ AF 3 for variability	0.005 respirable fraction for soluble nickel
AGS 2015	(Oller et al., 2008)	Lung inflammation	0.1 LOAEC	AF 3 LOAEC to NOAEC→ HEC→ AF 3 for variability	0.006 respirable fraction for nickel metal

Agency	Key study	Endpoint	Point of departure (mg Ni/ m³)	Assessment factors	OEL (mg/m³)
AGS 2017	(NTP, 1996a, b)	Lung tumors	0.491	HEC	0.013 4:1 000 0.006 4:10 000 0.001 4:100 000 for nickel compounds

Table IV.13: Additional occupational exposure limit values. Modified from (ECHA, 2018)

Country	Soluble nickel (mg/m³)	Insoluble nickel (mg/m³)	Nickel metal (mg/m³)
Austria	0.05 inhalable fraction for soluble nickel inorganic compounds	0.5 inhalable fraction for insoluble nickel inorganic compounds	0.5 for nickel metal
Belgium	0.1 inhalable fraction for soluble nickel inorganic compounds	0.2 inhalable fraction for insoluble nickel inorganic compounds	1 for nickel metal
Denmark	0.01 inhalable fraction for soluble nickel inorganic compounds	0.5 inhalable fraction for insoluble nickel inorganic compounds	
Ireland	0.1 for soluble nickel inorganic compounds	0.5 for insoluble nickel inorganic compounds	
Hungary	0.1 for soluble nickel inorganic compounds		
Finland	0.05 inhalable fraction and 0.01 respirable fraction for soluble inorganic nickel compounds	0.05 inhalable fraction and 0.01 respirable fraction for insoluble inorganic nickel compounds	0.01 respirable fraction for nickel metal
France			1 respirable fraction for nickel metal
Latvia			0.05 respirable fraction for nickel metal
The Netherlands	0.1 for soluble nickel	0.1 for insoluble nickel	0.1 for nickel metal
Romania	0.1 for soluble nickel	0.1 for insoluble nickel	0.1 for nickel metal
Spain	0.1 inhalable fraction for soluble nickel inorganic compounds	0.2 inhalable fraction for insoluble nickel inorganic compounds	1 for nickel metal
Sweden			0.5 for nickel metal
Switzerland	0.5 inhalable fraction for soluble nickel	0.5 inhalable fraction for insoluble nickel inorganic compounds	0.5 inhalable fraction for metallic nickel
United Kingdom	maximum exposure limit:	maximum exposure limit:	maximum exposure limit: 0.5 for metallic nickel

Country	Soluble nickel (mg/m³)	Insoluble nickel (mg/m³)	Nickel metal (mg/m³)
	0.1 inhalable fraction for soluble inorganic nickel compounds	0.5 for insoluble nickel inorganic compounds	
US-NIOSH	0.015 for soluble nickel inorganic compounds	0.015 for insoluble nickel inorganic compounds	0.015 for metallic nickel
US-OSHA	1 for soluble nickel inorganic compounds	1 for insoluble nickel inorganic compounds	1 for metallic nickel

Table IV.14: Additional reference values and occupational biomonitoring guidance values. Modified from (ECHA, 2018)

Country	Soluble nickel compounds	Insoluble nickel compounds	Notations
Finland	12 µg/L in urine	6 µg/L	
Germany	Range of values starting from value of 25 µg/L urine for an external concentration of 0.025 mg/m³ in air	Range of values starting from value of 15 µg Ni/l urine for an external concentration of 0.1 mg/m³ in air	EKA value: Exposure equivalents for carcinogenic substances, it shows correlation between internal and external exposure
Germany	3 µg/L in urine		BAR value: Background level of a substance which is present concurrently at a particular time in a reference population of persons of working age who are not occupationally exposed to this substance
Romania	15 µg/L in urine		Binding value, sampling at the end of the shift
Canada	1.1 µg Ni/L in blood		95th percentile of the measured pollutant concentration levels in the relevant matrix of the reference population.
Canada	4.4 µg Ni/L in blood		95th percentile of the measured pollutant concentration levels in the relevant matrix of the reference population.

13 Derivation of HBM-GVs for Nickel with regard to the workers

Apel et al. (2020) established three possible ways to set an HBM-GV: HBM-GV derivation from human data based on a relationship between internal concentrations and health effects, HBM-GV derivation based on a defined external TRV or on a defined occupational exposure limit (OEL) value, and derivation based on a critical effect observed in experimental animal studies.

There are limited data to derive HBM-GV for nickel based on human internal concentration of exposure biomarker and health effects. However, suitable occupational exposure limit values are available for the derivation of HBM-GV for workers. Thus, this approach can be used to derive nickel HBM-GV for workers.

14 Choice of the OEL value for the derivation of an HBM-GV for workers

The main exposure route for workers is inhalation, although some hand-to-mouth exposure might exist. Human and animal data have shown an increased risk of lung and nasal cancer after inhalation exposure. ECHA (2018), RAC (2018), and EFSA (2020) have determined nickel compounds to be indirect genotoxic carcinogens. This evaluation is supported by the most recent data made by Oller et al. (2023). RAC (2018) calculated an OEL value of 0.005 mg/m^3 for respirable particles that is suitable for nickel metal and for soluble and poorly soluble nickel. NTP studies (1996a, b, c) were used for the point of departure and for nickel sulfate NOAEC of 0.03 mg/m^3, nickel subsulfide LOAEC of 0.11 mg/m^3, and for nickel oxide LOAEC of 0.5 mg/m^3 was determined. In all the studies, the particle size was in the respirable range. Nickel sulfate NOAEC value was based on 2-year inhalation study and lung inflammatory effects (NTP, 1996c). The nickel subsulfide and nickel oxide LOAEC value was used as no NOAEC value was available in a 2-year inhalation study (NTP, 1996a, b). The LOAEC value was based on lung inflammatory, fibrosis and carcinogenic effects.

After using assessment factor for LOAEC to NOAEC conversion, and calculating HEC, (human equivalent concentration) the same NOAEC 0.03 mg/m^3 was reached for all three nickel compounds. For HEC, calculations were made on a multiple-path particle deposition model (MPPD, Asgharian et al., 1999). MPPD model predicts the deposition of particles in the alveolar and tracheo-bronchial region. Received NOAEC is the same as calculated by Oller et al. (2023) for the nickel subsulfide and oxide using the inflammation endpoint of a 2-year study after integrating the data from NTP studies (1996b, c) and from Efremenko et al. (2014, 2017) studies. For nickel metal, a 0.1 mg/m^3 LOAEC was used as the point of departure (Oller et al., 2008; RAC, 2018). A calculated NOAEC 0.02 mg/m^3 was received after using the assessment factor and calculating HEC. RAC (2018) stated that humans do not seem to be more sensitive than rats to the carcinogenic effects. Thus, an assessment factor of 1 was considered sufficient for toxicodynamics. For intraspecies variability, an assessment factor of 3 is applied and for the severity of the endpoint, an additional assessment factor of 2 was used for nickel compounds. However, as nickel metal has not shown carcinogenic potential, only an assessment factor of 3 for intraspecies variability was applied.

For inhalable fraction, RAC calculated an OEL value of 0.03 mg/m^3 using the Kristiansand cohort (Grimsrud et al., 2002; Grimsrud et al., 2003), as nasal cancer has been detected in epidemiological studies. RAC pointed out that this is a conservative approach due to the use of Haber´s rule in the conversion and the Kristiansand cohort has the highest risk estimates when compared to other epidemiological analysis.

In addition, RAC considered that the OEL value is also protective against reproductive effects based on the EFSA (2015) TDI value of 2.8 µg/kg bw/day. The newest TDI value of 13 µg/kg bw/day is also based on reproductive effects (EFSA, 2020). When the TDI value of 13 µg/kg bw/day is converted into inhalation exposure (using formula 3) 91 µg/m^3 is achieved. This value is well above the RAC proposed OEL value; thus, OEL value can be considered protective against reproductive effects also with the updated EFSA value.

$$inhalation\ exposure = \frac{concentration * body\ mass}{inhalation\ volume}$$

$$inhalation\ exposure = \frac{13\ \frac{\mu g}{kg\ bw/day} * 70\ kg}{10\ m^3/day} = 91\ \mu g/m^3$$

Formula IV.3: Oral exposure converted into inhalation exposure. Standard values for dose calculations: inhalation volume for workers (m^3/8 hours of light work) is 10 m^3/day and body mass is 70 kg (ECHA, 2012).

Overall, it can be concluded that the use of OELs determined by RAC can be chosen as the starting point for the derivation of HBM-GV for workers.

15 Derivation of an HBM-GV for workers

15.1 Soluble nickel compounds

For soluble nickel, there are eight linear regressions available (table IV.15). In addition, in 2010, DFG derived a correlation based on Tola et al. (1979), Nieboer et al. (1999), and Oliveira et al. (2000) (Angerer, 2010). They did not use the Bernacki et al. (1980) study because the ambient air concentrations at a maximum value of 16.4 µg/m^3 were too low to determine 0.05 mg/m^3, a concentration they wanted to extrapolate. They also discussed that the ambient air concentrations in the Bernacki et al. (1980) study were 10 times lower than Tola et al. (1979) study and concluded that the results were implausible. However, DFG later withdrew the EKA (Exposure Equivalent for Carcinogenic Substances) value for soluble compounds due to variability in nickel excretion (Nasterlack M et al., 2021). Kiilunen et al. (1997a) stated that the low correlation in their study could be due to low exposure levels when compared to previous studies. Also, in their study at nickel refinery no correlation was found, and the authors suggested that nickel urine concentration better reflects total exposure from all exposure routes rather than inhalation exposure (Kiilunen et al., 1997b). In addition, the authors stated that hand-to-mouth exposure is a relevant exposure route due to poor personal hygiene practices (Kiilunen et al., 1997a).

In the existing regression line studies, total dust from the breathing zone has been usually analyzed. The inhalable OEL corresponds better to total dust than the respirable OEL, thus inhalable OEL is used for deriving HBM-GV for workers. It should be noted that the personal air sampling method, which has been used in most of the epidemiological studies, uses 37 mm filter cassettes (ECHA, 2018). This method underestimates the captured nickel mass when compared to the IOM inhalable sampler as it does not capture the larger particles as efficiently. It has been noted that a correction factor of 2 should be used to account for this uncertainty (ECHA, 2018). This means that an inhalable limit 0.03 mg/m^3 corresponds to 0.015 mg/m^3 as total dust. The derived urine concentration ranged from 12.5-58.8 µg/L for soluble nickel exposure (Table IV.15). From the original linear regression lines, Oliveira et al. (2000) had the highest correlation. Using the linear regression equation from Oliveira et al. (2000), the urine concentration for soluble nickel exposure corresponding to RAC proposed inhalable OEL value of 0.03 mg/m^3 (which corresponds to 0.015 mg/m^3 as total dust (ECHA, 2018)), can be calculated: (6+430*0.015) = 12.45 µg/g creatinine corresponding to 16.9 µg/L. All conversions from µg/g creatinine to µg/L were achieved with the equation µg/L=µg/g creatinine * mean urine creatinine concentration in g/L (Santonen et al., 2023). Calculations were performed using a creatinine concentration of 1.36 g of creatinine determined by Cocker et al. (2011). The urine concentration would be even less than 16.9 µg/L with a lower creatinine concentration. When using the equation derived from the table provided by DFG, the following urine nickel concentration can be calculated (10+600 *0.015) =19 µg/L. Nieboer et al. (1999) equation resulted in a nickel urine concentration of 12.5 µg/L, Kiilunen et al. (1997a) resulted in 17 µg/L, Werner et al. (1999) resulted in 10.49 µg/g creatinine corresponding to 14.3 µg/L, and Ghezzi et al. (1989) resulted in 33.8 µg/L. It should be noted that in the Werner et al. (1999) study, respiratory protection was used in areas where air nickel concentrations exceeded the OEL value at that time. In addition, data from pre-shift and post-shift samples were pooled which might have affected the results (Werner et al., 1999).

At low exposure levels, variability in background nickel urine concentration levels might cause uncertainties in interpreting the results (OECD, 2022). Thus, urine concentration levels should not be interpreted at the individual level, but at the group level. The group level results should be compared to general population background levels (OECD, 2022). Hand-to mouth nickel exposure might affect urine nickel concentrations in addition to inhalation exposure but the extent of this effect is unknown. However, as the correlations are based on the measurement data it includes also the hand-to mouth exposure as the human biomonitoring data shows aggregated exposure, meaning that all exposure sources and routes are included (Santonen et al., 2023).

Due to uncertainties in the linear regression of low air nickel levels, the most conservative value was chosen. Based on these equations, **for workers, an HBM-GV of 12 µg/L is proposed for soluble nickel exposure.**

Table IV.15: Correlation lines between urine nickel and air concentrations after exposure to soluble nickel.

Linear regression	Air sample	n	r	Nickel air (µg/m³)	Workplace	Urine concentration corresponding to 0.03 mg/m³ (inhalable corresponds to 0.015 mg/m³ as total dust)	Reference
Ni (U) (µg/L) = 26.8 + 522.8 Ni (A) (mg/m³)	Total dust	19	0.82	30–160	Electroplating	34.6 µg/L	(Tola et al., 1979) information from (Angerer, 2010)
Ni (U) (µg/L) = 2.0 + 700 Ni (A) (mg/m³) based on data from late 1970s (no individual data available)	Total dust			Not available	Nickel refinery	12.5 µg/L	(Nieboer et al., 1999)
Ni total dust (U) (µmol/L) = 0.242 + 0.0036 (A) (µg/m³) Ni water soluble (U) (µmol/L) = 0.203 + 0.016 (A) (µg/m³)	Total dust	10 10	0.39 0.46	0.5–78.3	Electroplating	0.296 µmol/L (17.37 µg/L) 0.443 µmol/L (26 µg/L)	(Kiilunen et al., 1997a)
No correlation	Total dust			1.3–21	Nickel refinery	-	(Kiilunen et al., 1997b)
Ni (U) (µg/g creatinine) = 6.0 + 430 Ni (A) (mg/m³)	Total dust	13	0.96	2.8–116.7	Galvanizing plant	12.45 µg/g creatinine (16.9 µg/L)	(Oliveira et al., 2000)
Ni (U) (µg/L) = 6.3 + 3.5 Ni (A) (µg/m³)	Total dust	12	0.70	9.3 mean 4.7–16.4	Electroplating	58.8 µg/L	(Bernacki et al., 1980)

Linear regression	Air sample	n	r	Nickel air (µg/m³)	Workplace	Urine concentration corresponding to 0.03 mg/m³ (inhalable corresponds to 0.015 mg/m³ as total dust)	Reference
Ln Ni (U) (µg/g creatinine) = 4,73 + 0,677 *Ln (Ni (A)) (mg/m³).[A]	Inhalable	20	0.54 (r^2)	11.4–53.1	Nickel refinery	ln Ni=2.35 µg/g creatinine e^2.35=10.49 µg/g creatinine (14.26 µg/L)	(Werner et al., 1999)
Ln Ni U [µg/L] = 0.41 + 1.15 × ln Ni (A) (µg/m³)	Total dust	23	0.68	Two groups: > 10 (GM 11.2–18.8) < 10 (GM 4.2–6.9)	Electroplating	ln Ni=3.52 µg/L e ^3.52= 33.8 µg/L	(Ghezzi et al., 1989)
Ni (U) (µg/L) = 10 + 600 Ni (A) (mg/m³).[B]			0.99 (r^2)			19 µg/L	(Angerer, 2010)

A= Soluble nickel exposure estimates were obtained by multiplying nickel exposure estimates by a factor reflecting the prevalence of soluble nickel for total and inhalable aerosol in each of the four workplace refineries.

B= DFG presented a table for 3 different concentrations of nickel in urine and in air: 0.025 mg/m³ corresponds to 25 µg/L, 0.05 mg/m³ corresponds to 40 µg/L, 0.1 mg/m³ corresponds to 70 µg/L. Linear regression line is made from these points. DFG calculated values based on (Nieboer et al., 1999; Oliveira et al., 2000; Tola et al., 1979)

15.2 Sparingly soluble nickel compounds

Correlation equations between air nickel concentration and nickel urine concentration might be used to derive HBM-GV corresponding to the OEL value of 0.03 mg/m³. There are eight studies that have studied correlations between urine sparingly soluble nickel and nickel air (table IV.16). In addition, DFG has formed a correlation based on four studies (Emmerling et al., 1989; Rahkonen et al., 1983; Raithel, 1987; Sunderman et al., 1986a). However, it should be noted that DFG made an addendum in 2020 where they used six studies (Emmerling et al., 1989; Rahkonen et al., 1983; Raithel, 1987; Sunderman et al., 1986a, Thomassen et al. 1999, and Nieboer et al. 1999) (Nasterlack M et al., 2020). In the addendum, DFG concluded that the correlation cannot be extrapolated to the air concentration levels below 100 µg/m³ as the air concentration range was above 100 µg/m³ in all the studies (Nasterlack M et al., 2020). The Thomassen et al. (1999) study only shows equation lines based on previous studies and conforms these lines, thus, Thomassen et al. (1999) study is not considered in current derivation process. In addition, in the three most recent studies, no correlation between nickel air and sparingly soluble nickel urine concentrations were detected (Stanislawska et al., 2020; Stridsklev et al., 2004, 2007).

This inhalable OEL value of 0.03 mg/m³ corresponds to 0.015 mg/m³ as total dust. It can be noted that correlations were strong only when the nickel air concentration at the workplace was clearly higher than the inhalable limit value of 0.03 mg/m³. When workplace nickel air concentration levels were below the

inhalable limit value, the correlation was poor or non-existent. Hence, there seems to be significant uncertainty at lower air levels.

Urine concentrations for sparingly soluble nickel exposure, corresponding to the RAC proposed inhalable OEL value of 0.03 mg/m³ (=30 µg/m³), range from 3.3 µg/L to 14 µg/L (table IV.16). Calculations were done using linear regression equations.

There are significant uncertainties related to the air-urine correlations at low exposure levels. Using the most conservative regression equations, values of 3.3–4.5 µg/L can be calculated to correspond to air levels of 0.03 mg/m³. These values are very close to P95 of nickel urine concentrations from the EU general population, which typically range from 4.3 to 6 µg/L in non-polluted regions (Table IV.3). Thus, when exposed to sparingly soluble nickel, nickel urine concentrations should remain at the level of the general population. This means that nickel urine concentration should stay below the nationally or regionally defined reference values. **Therefore, no HBM-GV for sparingly soluble nickel can be recommended.** When assessing potential exposure to nickel using biomonitoring, the collection of paired samples has been recommended (RAC, 2018). In the paired samples both pre-shift and post-shift samples are taken from the same individual.

Table IV.16: Correlation lines between urine nickel and air concentrations after exposure to sparingly soluble nickel.

Linear regression	Air sample	n	r	Nickel air (µg/m³)	Workplace	Urine concentration corresponding to 0.03 mg/m³ (µg/L) (inhalable corresponds to 0.015 mg/m³ as total dust)	Reference
Ni U (µmol/L) = 0.08 + 0.8 Ni (A) (mg/L)	Total dust	10	0.95	150 mean	MMA stainless steel welders	4.7	(Rahkonen et al., 1983)
Ni U (µg/g creatinine) = 13.5 + 0.05 Ni (A) (µg/m³)	Total dust	22	0.52			14.3	(Raithel, 1987) information from (Angerer, 1995; ECHA, 2018)
Ni U (µg/L) = 10.84 + 0.007 Ni (A)(µg/m³)	Total dust	174	0.52	MMA 25 MAG 68.6 TIG 8	Stainless steel welders	10.9	(Emmerling et al., 1989) information from (Angerer, 1995)
Ni U (µg/L) = 8.49 + 56.88 Ni (A) (mg/m³)	Total dust	6	0.86	Drying of nickel 310 Production of nickel powder 310	Groups of different exposures	8.6	(Sunderman et al., 1986a) according to data by Morgan and Rouge. Information

Linear regression	Air sample	n	r	Nickel air (µg/m³)	Workplace	Urine concentration corresponding to 0.03 mg/m³ (µg/L) (inhalable corresponds to 0.015 mg/m³ as total dust)	Reference
				Contact with sparingly soluble nickel compounds 1540			from (Angerer, 1995)
Ni (U) (µg/L) = 2.0 + 85 Ni (A) (mg/m³) based on data from late 1970s (no individual data available)	Total dust				Nickel refinery	3.3 µg/L	(Nieboer et al., 1999)
No correlation	Total dust			18.92 mean	Stainless steel grinders	-	(Stridsklev et al., 2007)
No correlation	Total dust			50.4 mean	Stainless steel welders	-	(Stridsklev et al., 2004)
Not available	Inhalable and respirable dust		0.034	21.2 mean 8.5 median	MIG welders	-	(Stanislawska et al., 2020)
Ni U (µg/L) = 7.5+75 Ni (A) (mg/m³). [A]			1			8.6	(Angerer, 1995)

MMA= Manual metal arch welding, MAG= metal active gas welding, MIG= metal inert gas welding, TIG= tungsten inert gas welding

A= DFG presented a table for 3 different concentrations for nickel in urine and in air: 0.1 mg/m³ corresponds to 15 µg/L,0.3 mg/m³ corresponds to 30 µg/L, 0.5 mg/m³ corresponds to 45 µg/L. Linear regression line is made from these points. DFG calculated values based on (Emmerling et al., 1989; Rahkonen et al., 1983; Raithel, 1987; Sunderman et al., 1986a)

16 Level of confidence attributed to the derived HBM-GVs for workers

When deriving HBM GVs, the level of confidence in the derived HBM-GV should be estimated as high, medium, or low (Apel et al., 2020; OECD, 2022).

The level of confidence proposed here, is based on expert judgement about the reliability of the data and the calculation method used in the HBM-GV derivation. The level of confidence for soluble nickel HBM-GV for workers can be estimated as medium because the data from the relationship between the correlation from air data to urine concentration has uncertainties at low levels, and occupational exposure is often a mixture of soluble and sparingly soluble compounds.

The level of confidence for sparingly soluble nickel HBM-GV for workers can be estimated as low because there are limited data on the relationship between internal concentration and health effects, and the correlation from air data to urine concentration has uncertainties at low doses, and occupational exposure is often a mixture of soluble and sparingly soluble compounds.

17 Discussion

For the general population, a TDI value of 13 µg/kg bw/day recommended by EFSA in 2020, was used for the derivation of the HBM-GVs. This value is based on post-implantation loss in the offspring and an increase in dead pups (Park et al., 2012; SLI, 2000a, b). The main exposure route for the general population is oral. Reproductive effects were chosen as the critical effect over the carcinogenicity due to a lack of evidence for carcinogenicity after oral exposure (EFSA, 2020). The excretion of nickel is affected by food consumption, with less being excreted if nickel has been administered with food (Nielsen et al., 1999; Sunderman et al., 1989). The general population is mainly exposed through food and water, thus, when deriving HBM-GVs, excretion values when administered with food were considered.

The derivation was done using a urinary mass balance approach and PBPK modelling, resulting in wide deviation of results depending on whether parameters were from a fasting status or a non-fasting status. There also remained high uncertainty in extrapolation kinetic parameters for HBM-GV estimation from the study that has used soluble nickel compound whereas general population is exposed to nickel in food that is estimated to be in a complex bound organic form. This different form of nickel might have different kind of toxicokinetics that is causing the uncertainty. Uncertainties of differences in form of nickel and fasting status is estimated to cause a too high spectrum to be able to propose HBM-GV for general population and for use of EU-wide policy actions. Instead, it is proposed an orientation HBM-GV range of 3-11 µg/L which refers to non-fasting status only. This orientation HBM-GV range can be used for local decision making.

The general population P95 urine nickel concentrations have ranged from 4.3-6 µg/L in non-polluted regions (Table IV.3). Proposed orientation HBM-GV range is between 3 and 11 µg/L. However, in polluted industrial sites, the P95 urine concentrations have been even as high as 18 µg/L (Chellini et al., 2017). Indeed, in polluted regions nickel urine concentrations are above the orientation HBM-GV range. This means that there are local regions where exposure of nickel is so high that it might increase the risk of health effects if HBM measures have been examined from subjects in non-fasting status. Thus, local decision making might be needed to reduce the exposure of the general population at polluted industrial sites. It is noteworthy, that proposed orientation HBM-GV range is set based on conservative assumptions for example on excretion and TDI. TDI has been set by EFSA that has used conservative assessment factors for the derivation of TDI (EFSA, 2020). Excretion varies depending on the fasting status and nickel form.

For workers, the OEL value of 0.03 mg/m^3 for the inhalable fraction, as recommended by RAC in 2018, was used for the derivation of the HBM-GV. This value is based on nasal cancer studies from the Kristiansand cohort (Grimsrud et al., 2002; Grimsrud et al., 2003). For workers, two HBM-GV are proposed, based on the nickel compounds solubility and kinetic differences. Soluble nickel compounds are more readily absorbed from the lungs and excreted into the urine. When workers are exposed to soluble nickel compounds, an HBM-GV of 12 µg/L is proposed. Electroplaters and nickel refineries were two main occupations in the nickel urine and air concentration correlation equations for exposure to soluble nickel compounds. In the case of sparingly soluble nickel compounds, the main exposure source has been stainless-steel welding in the correlation equations. For workers exposed to sparingly soluble nickel compounds, no HBM-GV can be recommended as the correlations corresponding to 0.03 mg/m^3 for inhalable fraction are poor.

Additionally, derived value of 4.5 µg/L is very close to the P95 urine nickel concentrations of the general populations (Table IV.3). Therefore, urine nickel levels should stay below national reference values. To assess potential exposure to sparingly soluble nickel using biomonitoring, the collection of paired samples has been recommended (RAC, 2018). However, it should be noted that occupational exposure often involves a mixture of both soluble and sparingly soluble nickel compounds.

The orientation HBM-GV range for the general population is based on reproductive effects. It was calculated that the OEL value of 0.03 mg/m^3 is also protective against reproductive effects. The exposure route differs from that of the general population, although some hand-to-mouth exposure might exist. Even in this case, exposure varies from the general population as during occupational exposure, exposure occurs without food,

whereas the general population is exposed mainly with food. Food markedly decreases the excretion of soluble nickel compounds.

Thus, the HBM-GV for workers is also protective for reproductive effects. Moreover, orientation HBM-GV range seems to be in line with HBM-GV for workers as upper value of orientation HBM-GV range value is 11 µg/L that is close to HBM-$GV_{workers}$ for soluble nickel compounds. For sparingly soluble compound no HBM-GV was recommended for workers but the HBM-GV should stay below national reference values. The P95 of urine nickel concentrations in the different general populations have ranged from 4.3-6 µg/L in non-polluted regions that is near to lower value of orientation HBM-GV range value.

18 Factsheet HBM-GVs for nickel for the general population

FACTSHEET for Nickel for the general population			
Parameter	**note**	**Value / Descriptor**	**Comments**
Orientation HBM range value	1	3-11 µg/L	
Level of confidence	2	Low	
Status	3	Agreed	
Year of issue	4	2025	
EC-No.	5	231-111-4	
CAS No	6	7440-02-0	
CLP classification	7		
Molar mass	8	58.693 g/mol	
Identification exposure biomarkers	9		
Molar mass of biomarker(s)	11	58.693 g/mol	
Key studies for TK data, authors, year	12	(Nielsen et al., 1999; Sunderman et al., 1989)	
Type of study	13	Human	
Route of exposure	14	Oral administration	
Half-life of biomarker(s)	15	28 ± 9 hours	
Fraction urine excreted (Fue(3d))	16	0.02 (2 %)	
Derivation method	17	Based on animal POD	
Key study, Author(s), Year	18	(SLI, 2000a, b) (EFSA, 2020)	
Species	18	Male and female Sprague-Dawley rats	
Route/type of study	18	Oral, one- and two-generation reproductive toxicity	
Exposure duration	18	Chronic	
Critical endpoint	18	post-implantation loss in the offspring, increased dead pups	
POD Value	18	$BMDL_{10}$=1.3 mg/kg bw/day	
Assessment factors	19	100	
TRV	20	13 µg/kg bw/day	
Calculated orientation HBM range value	21	3-11 µg/L	Mass balance approach and PBPK modelling

19 Factsheet HBM-GVs for nickel for the workers

FACTSHEET for Nickel for the occupationally exposed adults			
Parameter	**note**	**Value / Descriptor**	**Comments**
HBM-GV_{worker} for soluble nickel	1	12 µg/L	
HBM-GV_{worker} for sparingly soluble nickel	1	Not recommended	
Level of confidence	2	Medium	
Status	3	Agreed	
Year of issue	4	2025	
CLP-INDEX-No.	5		
EC-No.	6	231-111-4	
CAS No	7	7440-02-0	
Molar mass	8	58.693 g/mol	
Molar mass of biomarker(s)	9	58.693 g/mol	
Key studies for TK data, authors, year	10	(Nielsen et al., 1999; Sunderman et al., 1989)	
Type of study	11	Human	
Route of exposure	12	Oral administration	
Half-life of biomarker(s)	13	28 ± 9 hours	
Derivation method	14	Based on correlation of human urine nickel concentration and air nickel concentration	
Key study, Author(s), Year	15	Kristiansand cohort (Grimsrud et al., 2002; Grimsrud et al., 2003) (RAC, 2018)	
Species	15	Human	
Route/type of study	15	Epidemiologic	
Exposure duration	15	Chronic	
Critical endpoint	15	Nasal cancer	
POD Value	15	0.04 mg Ni/ m^3	
Assessment factors	16	AF 3 LOAEC to NOAEC, a correction factor 2 for historical changes	
TRV	17	0.03 mg/m^3 for inhalable dust	OEL inhalable
Calculated HBM-GV_{worker}	18	12 µg/L for soluble nickel	

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Part V

Substance monograph for **diethylhexyl terephthalate (DEHTP)** and derivation of HBM guidance values (HBM-GVs) for the general population

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Responsible author(s)	Katharina Müller-Dott and Falko Partosch on behalf of UBA
Co-authors	Petra Apel/UBA/petra.apel@uba.de
Internal Reviewers[2]	WP4 co-leaders
External Reviewers[3]	Experts from NH Austria, NH Belgium, NH Swiss Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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PP = Restricted to other programme participants (including the Commission Services)

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Final version	May 2025	Review of AD 4.3 by EAA	Update of 24h bodyweight-related urine volume for adults and recalculation of HBM-GVs for adults. Supplemeting the abstract and introduction with more information on exposure. Removal of information on DNELs Consideration of further toxicity studies

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Abstract

Di(2-ethylhexyl) terephthalate (DEHTP), a terephthalate ester increasingly used as a plasticizer in various products, is serving as a substitute for toxicologically less favourable ortho-phthalates. DEHTP is predominantly utilized in PVC and other polymeric materials, with a presence of up to 30% in certain products. Concerns about its environmental and human health impacts are rising due to its increasing usage. Therefore, a systematic literature search was conducted at Pubmed and SCOPUS on DEHTP and its metabolites. Up to May 2023, 194 publications were found, of which 65 were considered relevant and were further evaluated. In addition, existing authority reports were evaluated and the limit values derived from them were taken into consideration.

Acute and repeated dose studies in animals indicated a rather low toxicity of DEHTP, carcinogenicity studies indicated limited effects, with the main exposure pathway assumed to be oral intake. It can be assumed that DEHTP is mainly ingested orally, as it is used as a substitute for toxicologically less favourable ortho-phthalates in consumer-related products such as toys, food contact materials and medical devices, through which they are known to be mainly ingested orally. With the increasing use of DEHTP, increasing exposures of the general population have been shown.

The urinary mass balance approach was used to derive HBM guidance values (HBM-GVs) for the major specific urine metabolite 1-Mono-(2-ethyl-5-carboxyl-pentyl) benzene-1,4-dicarboxylate (5cx-MEPTP) and another specific urine metabolite, 1-Mono-(2-ethyl-5-hydroxy-hexyl) benzene-1,4-dicarboxylate (5OH-MEHTP), which is excreted in smaller amounts. The HBM-GV for the sum of the two metabolites was also calculated.

A toxicity reference value (TRV) of 0.54 mg/kg bw/d based on the benchmark dose lower confidence limit $BMDL_{10}$ for effects on the retina in a chronic animal study, served as the basis for the HBM-GV derivation. As a result, HBM-GVs for women are 1.58 mg/L urine for 5cx-MEPTP, 0.209 mg/L urine for 5OH-MEHTP and 1.79 mg/L urine for the sum of the two specific metabolites 5cx-MEPTP and 5OH-MEHTP. For children and men, the HBM-GVs are 1.85 mg/L, taking into account the major specific metabolite 5cx-MEPTP, 0.244 mg/L for the specific metabolite 5OH-MEHTP and 2.09 mg/L for both metabolites[12].

Key Words

Human biomonitoring, human biomonitoring guidance values, HBM-GV, DEHTP, 5cx-MEPTP, 5OH-MEHTP

[12] By using the outdated 24h bodyweight-related urine volume for men and women of 0.02 L/(kg bw x d) for the calculation of HBM-GV, the resulting $HBM\text{-}GV_{GenPop\ (adults)}$ would have been 2.77 mg/L for 5cx-MEPTP, 0.37 mg/L for 5OH-MEHTP and 3.14 mg/L for the sum of the two specific metabolites 5cx-MEPTP and 5OH-MEHTP.

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Authors and Acknowledgements

We would like to thank the experts who provided us feedback on this work during the consultation period. Comments have been taken into account as far as possible. An overview of the substantive comments and how they were dealt with can be found in the annex to this deliverable comprising various reports.

Results

Health-based HBM guidance values (HBM-GVs) have been derived for the general population for two different urinary DEHTP metabolites. A TRV of 0.54 mg/kg bw/d based on a $BMDL_{10}$ for effects on the retina served as the basis for the HBM-GV derivation. As a result, HBM-GVs for women are 1.58 mg/L urine for 5cx-MEPTP, 0.209 mg/L urine for 5OH-MEHTP and 1.79 mg/L urine for the sum of the two specific metabolites 5cx-MEPTP and 5OH-MEHTP.

For children and men, the HBM-GVs are 1.85 mg/L, taking into account the major specific metabolite 5cx-MEPTP, 0.244 mg/L for the specific metabolite 5OH-MEHTP and 2.09 mg/L for both metabolites.

Abbreviations and Acronyms

BDI	Battelle Developmental Inventory
BMI	Body mass index
BMD(L)	Benchmark dose (lower confidence limit)
C	Carbon
CAS	Chemical Abstracts Service
CHO/HGPRT	Chinese hamster ovary/hypoxanthine guanine phosphoribosyl transferase
DEHP	Di(2-ethylhexyl) phthalate (bis(2-ethylhexyl)phthalate)
DEHTP	Di(2-ethylhexyl) terephthalate
DINP	Diisononyl phthalate
DNEL	Derived no effect level
DOTP	Dioctyl terephthalate
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EH	2-ethylhexanol
EPA	Environment Protection Agency
F_{ue}	Urinary excretion factor
GLP	Good Laboratory Practice
HBM	Human biomonitoring
HBM-GV	Human biomonitoring guidance value
IUPAC	International Union of Pure and Applied Chemistry
LD50/LC50	LD50 is the amount of a test substance, administered once, which causes the death of 50% of a group of test animals/LC50 means the substance concentration that kills 50% of the animals
LD, MD, HD	Dose levels: low dose, mid dose, high dose
MEHT	Mono-2-ethylhexyl terephthalate
MEHTP	Mono-2-ethylhexyl terephthalate
5cx-MEPTP	1-Mono-(2-ethyl-5-carboxyl-pentyl) benzene-1,4-dicarboxylate
2-cx-MMHTP	1-Mono-(2-carboxyl-methyl-hexyl) benzene-1,4-dicarboxylate
5OH-MEHTP	1-Mono-(2-ethyl-5-hydroxy-hexyl) benzene-1,4-dicarboxylate
5oxo-MEHTP	1-Mono-(2-ethyl-5-oxo-hexyl) benzene-1,4-dicarboxylate

NOAEL	No observed adverse effect level
NOEL	No observed effect level
OECD	Organisation for Economic Co-operation and Development
OECD SIDS	OECD Screening Information DataSet
OH	Hydroxy
POD	Point of departure
PVC	Polyvinylchloride
QSAR	Quantitative structure activity relationship
RfD	Reference dose
TG	Test guideline
TDI	Tolerable daily intake
TG	Test guideline
TPA	Terephthalic acid
TRV	Toxicological (Toxicity) reference value

1 Introduction

Terephthalates are esters of terephthalic acid (1,4-benzenedicarboxylic acid) and are increasingly being used as alternative plasticisers to the toxicologically less favourable phthalates. A well-known representative of terephthalates is di(2-ethylhexyl) terephthalate (DEHTP, bis(2-ethylhexyl) terephthalate) (ANSES 2015, 2016).

DEHTP is preferably used as a substitute for di(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DINP), for example for PVC in the food sector, for cellulose acetate butyrate, cellulose nitrate, polymethyl methacrylate, polystyrene, and polyvinyl butyral. The substance is used in the following products: polymers, coating products, adhesives and sealants. It can be present in up to 30 % in the products used. DEHTP consumption volumes have been rapidly increasing from 2000 metric tons in the year 2002 to 100,000 metric tons in the year 2014 and predicted 135,000 metric tons in 2019 (data for Western Europe, Malveda et al., 2015 cited in Lessmann et al., 2019).

Human biomonitoring studies in several European countries (Belgium, Denmark, Germany), the U.S., and Mexico, Asia and Saudi Arabia have shown that specific urinary metabolites of DEHTP were detectable in the general population and at increasing concentrations over time (Bastiaensen et al., 2021; Frederiksen et al., 2020; Lessmann et al., 2019; Silva et al., 2019; Wu et al., 2020; Ketema et al., 2023; Lee et al., 2021).

DEHTP was prioritized within PARC in the PARC Aligned Study Scope category.

The present report is intended to serve as an evaluation basis for human biomonitoring studies.

2 Methodology

Relevant agency reports and risk assessments for DEHTP were identified by general desk research. For a systematic literature search of scientific publications, a search string was developed consisting of (A) a health part and (B) synonyms for DEHTP and its major metabolites (Table V.15). The full search term is shown in the Appendix.

Table V.15: Parts of the search term used to search for scientific publications

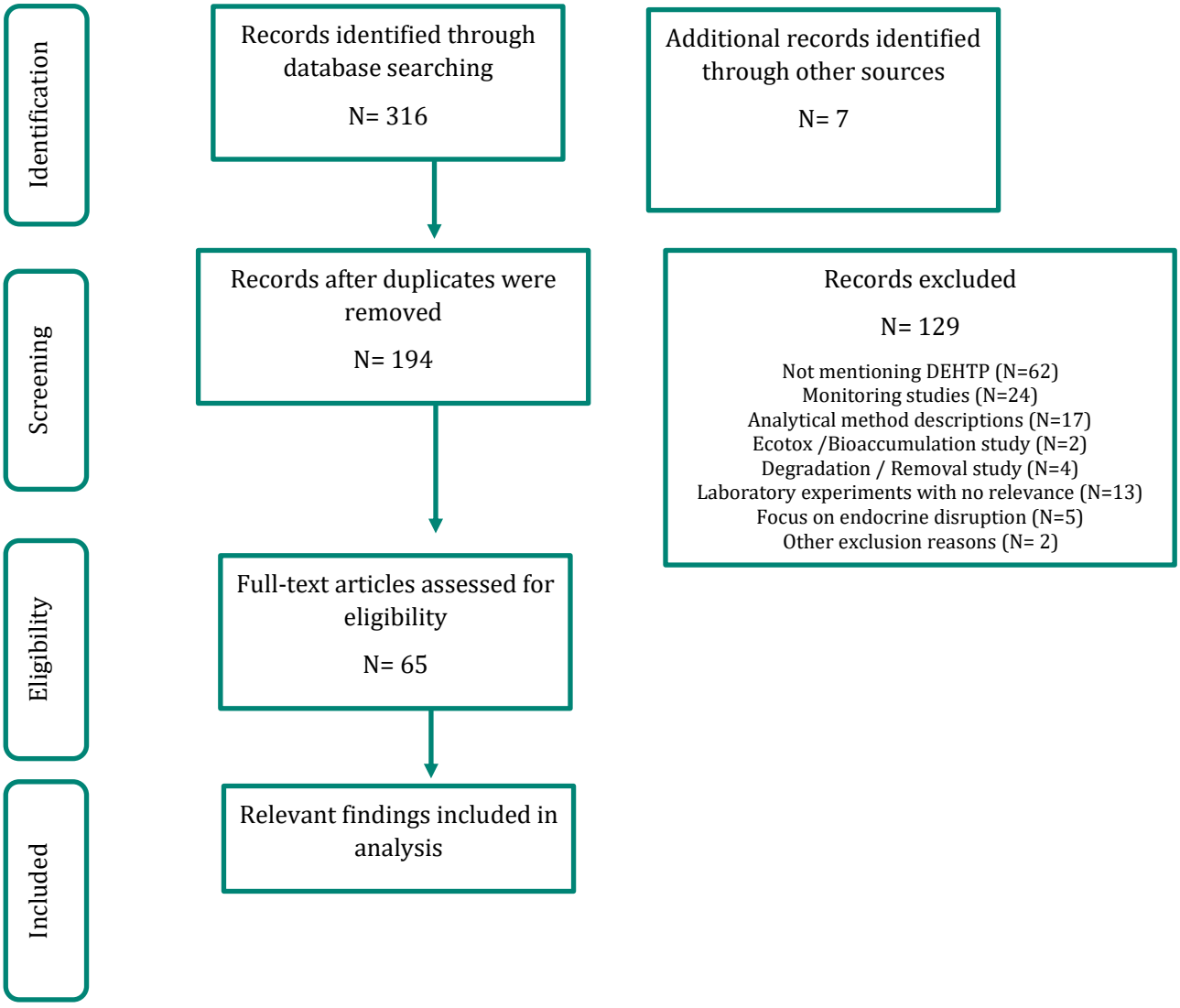
Health part	health OR adverse OR toxic* OR neurotox* OR expos* OR epidem* OR cohort OR genotox* OR genetic* OR carc* OR cancer OR reproduc* OR reprotox* OR developmental OR fertil* OR disease OR neurologic* OR metabol* OR ADME OR PBPK OR PBTK OR excret* OR “in vivo” OR "in vitro" OR "in silico" OR endocrine OR "half-life" OR immun* OR MOA OR "mode of action"
Synonyms for DEHTP and its major metabolites	DEHTP OR "Di(2-ethylhexyl) terephthalate" OR MEHTP OR “Mono-(2-ethylhexyl) terephthalate OR 5OH-MEHTP” OR “Mono(2-ethyl-5-hydroxyhexyl) terephthalate” OR 5oxo-MEHTP OR Mono(2-ethyl-5-oxo-hexyl) terephthalate OR 5cx-MEPTP OR “Mono(2-ethyl-5-carboxylpentyl) terephthalate “OR 2cx-MMHTP OR “Mono(2-carboxylmethyl-hexyl) terephthalate” [OR *additional synonyms of all substances*]

The search was carried out in SCOPUS and Pubmed and revealed a total number of 194 publications that were published until May 2023. The publications were screened for relevance according to title and abstract using DistillerSR. In this first step, 129 publications were excluded. The remaining 65 publications were screened by full text and relevant information is discussed in the following chapters of this report.

Exclusion criteria were the following:

- No information on DEHTP or its metabolites
- Study type not of interest (e.g. analytical methods, removal, laboratory experiments)
- Studies with no information relevant for the derivation of human biomonitoring values

The following PRISMA diagram shows the process graphically.



3 General characteristics

DEHTP is a colourless, viscous liquid and is used to soften PVC plastics and other polymeric materials (Barber, 1994). It is known for its chemical similarity to general purpose phthalates such as DEHP and DINP. Due to its very good plasticising properties, it can be used as a direct substitute for DEHP and DINP in many applications.

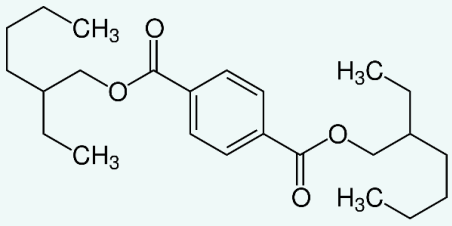
The volatility of DEHTP is low, it exhibits good softness at low temperatures, and its water, oil and electrical oxygen resistance are excellent (X. Weng et al. 2023).

According to the majority of notifications provided by companies to ECHA, in CLP notifications no hazards have been classified and DEHTP has no harmonized classification according to annex VI of the CLP regulation.

3.1 Physico-chemical properties

The information on physicochemical properties of DEHTP listed in Table V.16 is based on the registration dossier (ECHA 2023).

Table V.16: Substance characteristics and physico-chemical properties of DEHTP

Names	• Bis(2-ethylhexyl) terephthalate • Di(2-ethylhexyl) terephthalate • Benzene-1,4-dicarboxylic acid, bis(2-ethylhexyl) ester • Diethylhexyl terephthalate (DEHTP/DEHT) • Dioctyl terephthalate (DOTP)
IUPAC Name	• 1,4-bis(2-ethylhexyl)benzene-1,4-dicarboxylate
EC No.	229-176-9
CAS No.	6422-86-2
Sum formula	C_{24} H_{38} O_4
Structural formula	
Appearance [1013.25 hPa; at 20°C]	Liquid
Molecular weight [g/mol]	390.56 (Alves et al. 2017)
Relative density [g/cm³; at 20°C]	0.98
Melting point [°C; 1013.25 hPa]	< -67.2
Boiling point [°C; 1013.25 hPa]	375
Vapour pressure, Pa [at 25°C]	< 0.001
Log P_{OW} [25°C, pH 7], QSAR	7.81 – 9.54
Water solubility [µg/L; at 22.5°C, pH 5]	approx. 0.4 (Alves et al. 2017)
Dissociation constant [pK_a (= -log K_{10a})]	- 5.3
Henry Law's constant [Pa m³/mol]	1.21 (Alves et al. 2017)

3.2 Use of the substance

DEHTP occurs as a plasticiser in polyvinyl chloride (PVC) in a proportion of around 15 to 30% and is also used for materials in contact with food (see below). It is considered to be less harmful to health and is therefore used as a replacement for DEHP, which has been listed in the Restriction of Hazardous Substances Directive 2011/65/EU (ROHS) since 2015 due to its toxicological properties and is therefore subject to severe restrictions in use (X. Weng et al. 2023). DEHP is further identified as a substance of very high concern and subject to authorisation and restrictions[13] for specific uses within the REACH Regulation (Regulation (EC) 1907/2006). It is classified as toxic to reproduction (Repr. 1B; H360FD) according to CLP.

It is found in bottle caps, pipes, conveyor belts, sealing gaskets, shoe soles, pipe and cable coverings, floor coverings, gaskets used for bottle caps and waterproof clothing. It is widely used as a material for food contact, toys, medical equipment, flooring or cable insulation and is used as an alternative to banned phthalates (X. Weng et al. 2023) (OECD SIDS 2003).

According to the OECD SIDS Initial Assessment Report, DEHTP is slowly released into the environment during various uses of PVC (OECD SIDS 2003).

3.3 Possible route of exposure

The German Federal Institute of Risk Assessment (BfR) has estimated several sources of exposure to phthalates using the example of DEHP (UBA 2012). According to the evaluation, consumers mainly ingest DEHP orally with the main source of intake originating from food. DEHP was selected as representative for all phthalates. It is therefore assumed that the primary exposure pathway for DEHTP is also oral exposure. This is exemplified in a study by Edwards et al. (2022) on phthalate and novel plasticizer concentrations in food items from U.S. fast food chains, where it was shown that DEHTP was abundant in the food samples and was the most frequently detected chemical.

Releases of DEHTP to the environment are likely from indoor use (e.g. machine wash/cleaning products, car care products, paints and coatings or adhesives, fragrances and air fresheners) including indoor use in durable materials with a low release rate (e.g. floor coverings, furniture, toys, building materials, curtains, shoes, leather products, paper and cardboard products, electronic devices), and outdoor use in durable materials with a low release rate (e.g. construction and building materials made of metal, wood and plastic)[14].

Human exposure to vapours is considered unlikely due to the low vapour pressure which is estimated to be 2.85 E-5 hPa at 25° C. Under heated conditions with a temperature of 270 °C the vapour pressure was still only 13.3 hPa (OECD SIDS 2003). It is further stated that a potential for direct dermal exposure is created by coated fabrics where a flexible vinyl coating is applied on the surface to make them waterproof. However, systemic exposure via the dermal route is reduced, as demonstrated by the fact that DEHTP has an extremely low percutaneous absorption rate of 0.103 ± 0.052 µg/cm²/hr. Further exposure may result from the presence of this substance in the environment. However, air and water concentrations are expected to be low based on the limited vapour pressure and water solubility (OECD SIDS 2003). These statements were adopted by ANSES in its evaluation (ANSES 2015, 2016) and by the US Consumer Product Safety Commission (Risk Science Center 2018). In a more recent study, high DEHTP concentrations in house dust were associated with significantly higher urinary DEHTP metabolite levels for children in the German Environmental Survey GerES V from 2014 to 2017 (Schwedler et al. 2020).

The European Food Safety Authority (EFSA) evaluated DEHTP in 2008 and derived a tolerable daily intake (TDI) of 1 mg/kg bw/d based on a carcinogenicity study with a NOAEL for non-neoplastic lesions of 79 mg/kg bw/d (EFSA 2008) (see table V.3).

[13] https://echa.europa.eu/substance-information/-/substanceinfo/100.003.829
[14] https://echa.europa.eu/de/substance-information/-/substanceinfo/100.026.524

4 Toxicological reference values proposed by European or relevant non-European institutions

The following table V.3 summarises available toxicological reference values.

Table V.17: Available toxicological reference values

Agency	Type of value	Value	Key study	Exposure route	Critical effect	POD	AFs
EFSA	TDI	1 mg/kg bw/d	Unpublished data, (Deyo, 2008) assumed	Oral (diet)	Effects on the retina and the nasal turbinates	NOAEL: 79 mg/kg bw/d (males)	Not stated (default uncertainty factor of 100 is assumed, value was rounded up)
German HBM Commission	TDI	0.54 mg/kg bw/d	Deyo, 2008	Oral (diet)	Effects on the retina	$BMDL_{10}$: 54 mg/kg bw/d (BMD_{10}: 70 mg /kg bw/d)	A factor of 10 each to account for intraspecies and interspecies variability

5 Toxicological profile

5.1 Findings in humans

5.1.1 Toxicokinetics and metabolism

The metabolism and urine excretion kinetics of DEHTP were studied in three male volunteers after a single oral dose of 50 mg DEHTP. The study design was in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments. Approval for the study protocol was given by the responsible ethics committee and written informed consent was obtained from all individual participants included in the study (Lessmann et al. 2016). The predominant specific metabolite in urine was 1-mono-(2-ethyl-5-carboxyl-pentyl) benzene-1,4-dicarboxylate (5cx-MEPTP), which accounted for approximately 13.0% of the administered dose, followed by 1-mono-(2-ethyl-5-hydroxy-hexyl) benzene-1,4-dicarboxylate (5OH-MEHTP) (mean: 1.8%) and 1-mono-(2-ethyl-5-oxo-hexyl) benzene-1,4-dicarboxylate (5oxo-MEHTP) (mean: 1.0%). The metabolite 1-mono-(2-carboxyl-methyl-hexyl) benzene-1,4-dicarboxylate (2cx-MMHTP) was negligible, accounting for just 0.3% (range 0.3 - 0.4%). Considering the specific metabolites mentioned above, roughly 16.1% of the dosage was recovered in the urine within 48 hours, with the bulk (95%) expelled within the first 24 hours. Non-specific metabolites like TPA, which is the major metabolite of DEHTP, were not considered further. Therefore, the total recovery of the substance in urine is not given.In addition, it is known from toxicokinetic studies in animals, that a considerable amount of DEHTP is excreted in the faeces. However, the dose, which the three volunteers received, consisted of unlabelled DEHTP. Hence, it cannot be clearly distinguished between the exposure in this study and the regular background exposure. Nevertheless, quantification was attempted to be achieved by comparing pre-dose urine samples with post-dose urine samples, where the post dose metabolite levels were several orders of magnitude higher than levels observed in the pre-dose samples (pre dose: 5cx-MEPTP level at 1.4 µg/L, with the other three metabolites below the respective LOQs (0.2–0.4 µg/L), post dose: 86.7, 50.2, 923 and 21.5 µg/L for 5OH-MEHTP, 5oxo-MEHTP, 5cx-MEPTP and 2cx-MMHTP, respectively) (Lessmann et al. 2016).

There is also a human PBPK model available that was recently reported on (McNally et al. 2023). This was developed on the basis of an existing model to di(2-propylheptyl) phthalate (DPHP) with minor adaptions and model parameterisation based upon in silico and in vitro experimental data. However, McNally et al. (2023) consider the current validation of the model, which is fully parameterized with in vitro and in silico derived parameters, to be insufficient and consider it necessary to calibrate this model using multiple data streams from human biomonitoring to provide confidence for future assessments. At the present time using the read-across approach should be undertaken with caution even when the structure of the model itself is appropriate.

Therefore, the toxicokinetic data are used to derive the HBM-GVs in the following, instead of the PBPK data.

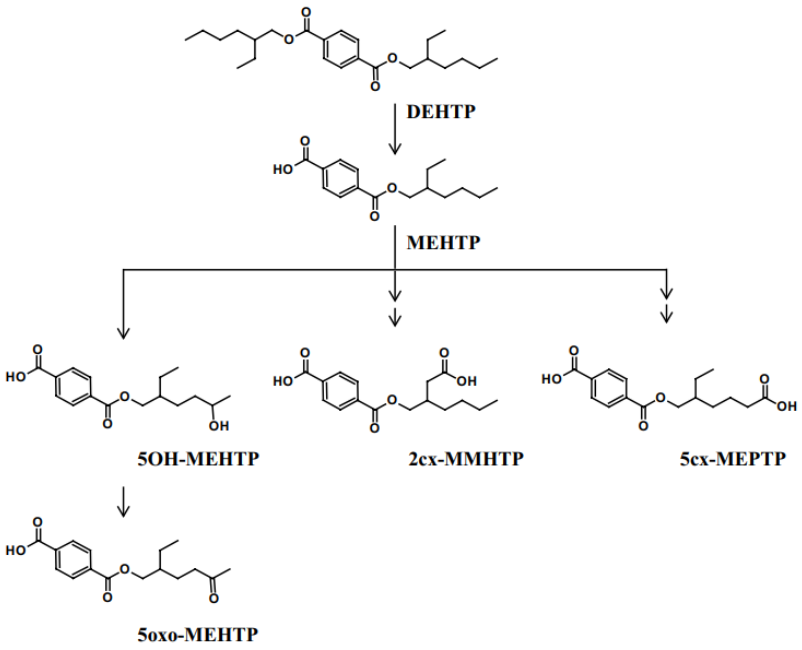


Figure V.2: Metabolic pathway of DEHTP, Source: (Lessmann et al. 2016), MEHTP: Mono-2-ethylhexyl terephthalate (278,34 g/mol), 5OH-MEHTP: 1-Mono-(2-ethyl-5-hydroxy-hexyl) benzene-1,4-dicarboxylate (294,33 g/mol), 2-cx-MMHTP: 1-Mono-(2-carboxyl-methyl-hexyl) benzene-1,4-dicarboxylate (308,32 g/mol), 5cx-MEPTP: 1-Mono-(2-ethyl-5-carboxyl-pentyl) benzene-1,4-dicarboxylate (308,32 g/mol), 5oxo-MEHTP: 1-Mono-(2-ethyl-5-oxo-hexyl) benzene-1,4-dicarboxylate (292,33 g/mol).

Study summaries and further information on human biomonitoring and toxicokinetic studies are given in Table V.18 of the Appendix.

5.1.2 Effects on skin and mucous membrane

In four studies with male and female volunteers, skin irritation and sensitisation were evaluated after DEHTP exposure (semi-occlusive conditions). In one study, only minimal irritation was observed, which was not associated with the substance, as the effect was not dose-dependent (Lockhart 2001a) as cited in (OECD SIDS 2003; ANSES 2015, 2016). The second study showed that DEHTP is non-irritating and non-sensitising to humans (David, Lockhart, and Ruble 2003). The third and fourth study confirmed that DEHTP appeared to be non-irritating and non-sensitising in all subjects (Lockhart 2001b and c). In summary, there are no indications, that DEHTP may have skin irritating or sensitising effects in humans.

Study summaries and further information are given in Table V.19 of the Appendix.

5.1.3 Carcinogenicity

In a case-control study in women in childbearing age (102 women from Korea, with 32 cases and 70 controls based on gynaecological examinations from 2015 to 2016), the association between DEHTP exposure and development of uterine fibroids was investigated. The odds ratio for uterine fibroids was significantly increased with DEHTP exposure, among other things; the metabolite OH-MEHTP was detected in 40.6 % of cases, compared to 29.1 % in the controls (Lee et al. 2020).
In a second case study, a connection between DEHTP exposure and leiomyomas of the uterus was investigated. The patients of the so called ELENA study consisted of 97 patients, including those with leiomyoma (n = 45), leiomyoma with endometriosis (n = 29), leiomyoma with adenomyosis (n = 10), and leiomyoma with endometriosis and adenomyosis (n = 13). Of these, 30 patients were randomly selected from whom samples of both leiomyomas and adjacent normal myometrial tissue were taken. In 70 % of the patients with leiomyoma, 12 phthalate metabolites and 3 DEHTP metabolites (5cx-MEPTP, 5OH-MEHTP, and 5oxo-MEHTP) were detected and 5oxo-MEHTP showed

positive association with methylation of oestrogen receptor ESR1 in leiomyoma (Cho et al. 2021). Both studies have several weaknesses and the respective authors are in favour for further research.

No further studies on carcinogenicity in humans are available.

Study summaries and further information are given Table V.20 of the Appendix.

5.1.4 Reproductive toxicity

Effects on Fertility

There are two studies available dealing with effects on fertility in humans. In one study, an association between urinary phthalate metabolites, serum sex hormones and relevant covariates was investigated. While premenopausal women had higher DEHTP concentrations than postmenopausal women, DEHTP was not statistically significantly associated with altered testosterone or oestradiol levels but was associated with a higher testosterone to oestradiol ratio in postmenopausal women which might be evidence that DEHTP may also be hormonally active (Long et al. 2021). In a second study with pregnant women, a positive correlation was established between the sum of DEHTP metabolites and the sum of oestrogens (Pacyga et al. 2023; 2021). However, according to the authors, the studies have some weaknesses and further investigation is needed for a reliable conclusion.

Table V.21 of the Appendix.

Developmental toxicology

A single study investigating developmental effects of DEHTP in humans is available. In this study, the association between gestational exposure to phthalates and their substitutes and neurodevelopmental disorders was investigated by Park and colleagues using a prospective birth cohort. A total of 274 mother-child pairs from Puerto Rico from the 14th week of pregnancy were analysed in the study by testing the mothers' urine for corresponding phthalate metabolites during pregnancy. The neurological development of the children was assessed at 6, 12 and 24 months of age using the Battelle Developmental Inventory (BDI) test, which assigns points for adaptive, personal-social, communication, motor and cognitive domains. Confounders such as age, education and BMI of the mothers, marital status, smoking habits, alcohol consumption, age, weight and gender of the children were eliminated.

In total, the concentrations of 15 phthalate and two DEHTP metabolites were measured. DEHTP metabolites were found to have significant associations in boys for the adaptive and cognitive domains at 6 months of age. At the age of 24 months, however, the motor areas were negatively affected. Girls, on the other hand, were only affected in communication at six months. According to the authors, the results show that exposure during pregnancy can affect several cognitive domains, with clear dependencies on the age and gender of the children, but further studies are needed to clarify long-term consequences and mechanisms (Park et al. 2023).

5.1.5 Association of exposure to DEHTP and its metabolites with nonalcoholic fatty liver disease (NAFLD)

Li et al. (2024) analysed data of the 2017−2018 National Health and Nutrition Examination Survey and found a positive linear correlation between the concentrations of the two DEHTP metabolites 5OH-MEHTP and 5cx-MEPTP (present in the urine samples of nearly all representative U.S. adults) and the risk of nonalcoholic fatty liver disease (NAFLD) in the population. Results of weighted quantile sum and Bayesian kernel machine regression indicated that 5OH-MEHTP contributed a greater weight to the risk of NAFLD in comparison with 12 conventional phthalate metabolites. Additional *in vitro* experiments with hepatocyte HepG2 revealed that 5OH-MEHTP exposure could increase lipogenic gene programs, thereby promoting a dose-dependent hepatic lipid accumulation. These findings provide new insights into the liver lipid metabolism toxicity potential of DEHTP exposure in the population.

5.2 Animal findings and *in vitro* studies

5.2.1 Toxicokinetics and metabolism

Several toxicokinetic studies have been performed.

The results of an *in vitro* experiment with human skin samples according to OECD 428 show that skin absorption of DEHTP is very low with an absorption rate through dermatomed skin of 0.103 ± 0.052 µg/cm²/h (Guerin et al. 2002 as cited in OECD SIDS, 2003). In an *in vitro* study using rat intestinal homogenate fractions, DEHTP was completely hydrolysed in the intestine to terephthalic acid (TPA) and 2-ethylhexanol (2-EH) (Barber et al., 1994). The bioavailability of DEHTP after inhalation was investigated *in vitro* using artificial lung fluid (artificial lysosomal fluid (ALF, pH 4.5) and Gamble's solution (pH 7.4)). The bioavailability decreased with increasing molecular weight and LogP of the phthalate esters analysed. The bioavailability of DEHTP was found to be very low in the test. Only 4.9 % could be detected in Gamble's solution and only 4.6 % in ALF (Kademoglou et al. 2018).

After oral administration to rats, most of the DEHTP which was partly ^{14}C-labeled was excreted in the faeces (56.5%) and urine (31.9%), with smaller amounts excreted in the exhaled air (3.6%). The majority of the material was detected in the faeces in the form of unchanged DEHTP (36.6% of the total dose), and 50.5% of the total dose (77% of the absorbed dose) was detected as unlabelled terephthalic acid (TPA) in the urine. A further 3.6% of the dose was excreted as CO_2 (OECD SIDS 2003).

Eight metabolites were reported and determined in urine: Terephthalic acid, mono-(2-ethyl-4-carboxypropyl) terephthalate, mono-(2-(carboxymethyl)-hexyl) terephthalate, 2-ethylhexanol, 2-ethylhexanoic acid, 5-hydroxy-2-ethylhexanoic acid, 5-hydroxy-2-ethylhexanlactone and 2-heptanone (ECHA 2023).

Study summaries and further information are given in Table V.22 of the Appendix.

5.2.2 Acute toxicity

Acute toxicity testing of DEHTP in laboratory animals shows that the substance has a very low potential for acute toxicity. No increased mortality was observed in any of the studies up to the highest doses administered.

Acute toxicity after oral exposure resulted in a LD50 of greater than 5,000 mg/kg bw in rats and >3,200 mg/kg bw or even 20,000 mg/kg bw in mice. For the dermal route of exposure a LD50 of greater than 19,670 mg/kg bw was established and for intraperitoneal exposure LD50 values greater than 3,200 mg/kg bw.

Data on inhalation exposure are not available due to the very low vapor pressure.

Study summaries and further information are given in

Table V.23 of the Appendix.

5.2.3 Studies with repeated exposure

The toxicity of DEHTP was investigated in several studies with repeated exposure (10 days – 2 years). Eight studies were performed in rats, one in guinea pigs, in four studies the route of exposure was oral via diet, in three studies the route of exposure was inhalation, and there was also a study for each cutaneous and intravenous route of exposure

There was no increased mortality in any study. No signs of toxicity were observed in the studies with inhalation or intravenously route of exposure and no significant adverse effects in the dermal study.

During a 90-day study in rats being exposed to DEHTP via the diet (Barber and Topping, 1995), the only effect being observed was increased liver weight for males and females of the high concentration group of 1%, corresponding to 561 and 617 mg/kg bw/d for male and female rats, respectively. Therefore, the NOAEL was set to 0.5%, which equals to 277 and 309 mg/kg bw/d, respectively (Barber and Topping, 1995).

The most relevant study is a GLP compliant 2-year chronic toxicity/carcinogenicity study by Deyo (2008) according to EPA guideline OPPTS 870.4200 with 50 F344 rats/sex/dose being exposed to 0, 1500, 6000, 12000 ppm, corresponding to 0, 79, 324, 666 mg/kg bw/d for males and 0, 102, 418, 901 mg/kg bw/d for females via the diet. There was no increased treatment related mortality and haematology, blood chemistry and urinalysis were without relevant treatment related findings. Adverse effects were statistically significant decreased body weight gains in mid and high dose groups. Body weight gain of males and females receiving 12,000 ppm was statistically significantly lower than controls at all intervals (0–16, 0–52 and 0–104 weeks). The body weight gain of males and females receiving 6000 ppm was statistically significantly lower than controls during the first year of the study (weeks 0–52), at the end of the study, the overall body weight gain of animals receiving 6000 ppm was similar to that of the controls. DEHTP had no effect on body weight or weight gain at 1500 ppm at any time.

At the end of the treatment period, the relative liver and kidney weights to body weights of high dose females were statistically significantly higher than control. The relative liver weight of high dose males (4.194 ± 0.920) also was higher than control (3.843 ± 0.759), but the difference was not statistically significant. Absolute kidney weights were significantly decreased in high and mid dose males and females, but were histologically without any significant increases in kidney lesions . Body weight and organ weight changes were also observed in other oral studies (e.g. Topping et al., 1987; Eastman Kodak Co., 1975) . Adverseeffects for the derivation of the NOAEL were non-neoplastic lesions in females of the mid and high dose groups like increased incidences of atrophy of the outer nuclear layer (ONL) of the retina and eosinophilic inclusions in the nasal turbinates. According to the author (Deyo, 2008), the study data do support the conclusion that at higher doses retinal ONL degeneration may have been exacerbated by DEHTP. A NOEL was established for this effect at the middle dose of 6000 ppm for males (324 mg/kg bw/d) and 1500 ppm (102 mg/kg bw/d) for females as the severity scores were identical to control at these exposure levels. The increased incidence of eosinophilic inclusions in the nasal turbinates of mid and high dose females was considered to be an exacerbation of an age-related finding and not of any toxicological significance.

According to EFSA (2008), however, both the effects on the retina and on the nasal turbinates are to be considered adverse and a NOAEL of 79 mg/kg bw/d was established, from which a rounded TDI of 1 mg/kg bw/d was derived.

Overall, the toxicity after repeated administration in different species following different routes of exposure is low (see Table V.10 of the Appendix).

5.2.4 Effects on skin and mucous membranes

Skin irritation was tested in a study according to OECD test guideline (TG) 404 in rabbits with no erythema or oedema being observed under occlusive conditions for 4 hours followed by an observation period of 72 hours (Product Safety Laboratories, 2006; cited by (ANSES 2015, 2016; Ball et al., 2012). Guinea pigs being exposed up to 19,680 mg/kg bw for 24 hours did show effects without clear dose dependencies (Eastman Kodak Co. 1975) as reported by (Versar, 2010; Ball et al., 2012)).

The potential of eye irritation was investigated in several studies with rabbits. In a study according to OECD TG 405 slight irritation was observed which turned into slight erythema within one hour and was reversible after

48 h (ECHA, 2012, cited in (ANSES 2015, 2016; ToxServices 2012). These effects were confirmed by other studies. It was concluded in the OECD SIDS document, that the substance might cause slight eye and skin irritation (OECD SIDS 2003). The potential of skin sensitization was investigated in several studies in guinea pigs. No evidence of an induction of contact sensitization was observed. According to (ANSES 2015, 2016), DEHTP does not need to be considered as a skin sensitizer.

Study summaries and further information are given in Table V.25 and Table V.26 of the Appendix.

5.2.5 Genetic toxicity

DEHTP does not contain any substructures commonly associated with mutagenicity; however, the genotoxic properties of DEHTP are of interest due to its isomeric relationship with DEHP. Different *in vitro* assays have been performed. The substance was tested in an Ames test with the *Salmonella* strains TA1535, TA1537, TA1538, TA98 and TA100 in concentrations of 1 to 10000 µg/plate, in an *in vitro* mutagenicity test on mammalian cells of the Chinese hamster ovary/hypoxanthine guanine phosphoribosyl transferase (CHO/HGPRT) at concentrations of 1.25 to 20 nl/ml, and an *in vitro* chromosome aberration test with CHO cells at concentrations of 700 - 1000 nl/ml. All tests were carried out with and without metabolic activation. All tests were negative (Barber, 1994).

DEHTP was administered to rats at doses of up to 2000 mg/kg bw/d over a period of 15 days. The rats' urine was then tested in an Ames test with the *Salmonella* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without S9 mix. The results were consistently negative.

There are no reports of *in vivo* studies (ECHA 2023). Study summaries and further information are given in Table V.27 of the Appendix.

5.2.6 Carcinogenicity

There are two reports of oral carcinogenicity studies conducted in F-344 rats. There were no signs of carcinogenic effects in a 104-weeks study up to 12,000 ppm, corresponding to 666 and 901 mg/kg bw/d for male and female rats, which is the highest dose tested (Deyo, 2008) as cited in (ANSES 2015, 2016; CHAP 2014; Risk Science Center 2018). In the second study, transitional cell adenomas of the urinary bladder were described but according to the Danish EPA, the study is negative for carcinogenicity. No primary information of the study is available. Due to the assessment of the authority, the tumours in the urinary bladder are assigned only minor significance. The NOAEL is 1000 mg/kg bw/d, i.e. the highest dose tested (Risk Science Center 2018).

Study summaries and further information are given in Table V.28 of the Appendix.

5.2.7 Reproductive toxicity

Fertility

Effects on fertility were investigated in a 2-generation reproductive toxicity study in rats according to OECD TG 416 (Faber et al., 2007b, Stump 2001b). There were no adverse effects observed on any reproductive parameter, litter parameters or spermatogenic endpoints in both generations. The NOAEL for reproductive toxicity was 1.0% in the diet (447-614 mg/kg bw/day for males and 595-1349 mg/kg bw/day for females), the highest dose tested. In addition to decreased body weights for different generations and life stages at higher doses, changes in organ weights were observed for F0: decreased absolute pituitary gland weights in HD males and increased relative liver weights in MD and HD females, F1: decreased lower mean absolute liver (-14.7%), kidney (-11.5%), spleen (-13.4%), testes (-7.9 and -10.4%) and adrenal gland (-14.6%) weights in HD males, relative weights were unaffected, spleen weights also decreased in LD and MD males, increased brain weights in HD males, decreased absolute kidney and uterus weights in HD females, increased relative brain, liver, spleen, and thymus gland weights in HD females. No histopathological changes were observed.In an *in vitro* assay (Rajkumar et al., 2022), Sertoli cells and spermatogonial cells were exposed to concentrations up to 100 µM. No effects were observed at any concentration.

While in the OECD TG 416 study ovarian follicle counts for the F(1) females in the high-exposure group were similar to the control values, recent research in mice indicate that DEHTP exposure disrupts folliculogenesis (effects at

100 µg/kg bw/d). Further studies are needed to clarify the contradictory results.Study summaries and further information are given in Table V.29 of the Appendix.

Developmental toxicology

Developmental toxicity of DEHTP was evaluated in nine studies in rats, mice and rabbits. Four studies were prenatal developmental toxicity studies according to OECD TG 414 in mice and rats and one was a two-generation developmental toxicity study according to OECD TG 416 in rats. Routes of exposure were oral via gavage or via the diet. In the animals being exposed, there were no signs of increased foetal mortality, visceral malformations or skeletal malformations even when accompanied by signs of maternal toxicity. However, in two studies increased incidences of pups with a 14th rudimentary rib were observed which is rather a variation than a malformation and therefore not considered adverse. DEHTP had no antiandrogenic effects in rats at 750 mg/kg bw/d (Gray et al., 2000). No alterations of spermatogenic endpoints or developmental landmarks were noted in the dietary OECD TG 416 study in rats. No effects on developmental toxicity were also observed in the other studies.

Study summaries and further information are given in Table V.30 of the Appendix.

6 Derivation of human biomonitoring guidance values

The purpose of HBM studies is to identify and quantify chemicals of concern or their metabolites in human biological material with a subsequent interpretation of the measurements to determine if (further) chemical management measures or regulation are needed (Apel et al. 2020).

HBM-GVs are guidance values that correspond to internal exposure levels at which no health impairment is expected according to current state of knowledge and considering only the individual substance.

6.1 Choice of the critical effect and POD

According to (Apel et al. 2020), there are three options to derive the HBM-GVs. The first option is to choose the POD according to the critical effect from human data, the second option is to use a defined external TRV, proposed by a European or relevant non-European institution and the third option is a POD based on a critical effect observed in experimental animal studies. Since no adequate human data is available, the second and third option is to be checked.

Recent studies on the toxicity of DEHTP and their results are not sufficient to question previous assessments. Studies examining reproductive parameters are contradictory. Therefore, in line with EFSA (2008), effects on the retina observed in mid and high dose Fischer 344 rats in a GLP compliant 104 week chronic toxicity/carcinogenicity study according to EPA guideline OPPTS 870.4200 are regarded as relevant with a NOAEL of 1500 ppm, corresponding to 79 and 102 mg/kg bw/d for males and females, respectively (Deyo, 2008). Toxicological reference values as listed in Toxicological reference values proposed by European
or relevant non-European institutions

The following table V.3 summarises available toxicological reference values.

Table V.17 refer to this key study. In 2008, EFSA derived on this basis an individual TDI of 1 mg/kg bw/d.

HBM-I values for adults and children were previously derived by the German HBM Commission (Apel et al., 2017). HBM-I values represent the concentration of a substance in human biological material up to which – according to the knowledge and judgement of the HBM Commission and taking into account only the single substance – there is no risk to be anticipated for adverse health effects. In the derivation of the German HBM Commission, the TDI of 1 mg/kg bw/d derived by EFSA was not used as TRV but a value of 0.54 mg/kg bw/d, which is based on a $BMDL_{10}$ of 54 mg/kg bw/d. A factor of 10 each to account for intraspecies and interspecies variability was selected to receive this TRV as in the derivation of a TDI. The $BMDL_{10}$ was based on degenerative effects on the outer granular layer of the retina of female rats from the study by (Deyo 2008) and was already used by NSF International (2012) for the derivation of an oral RfD. The corresponding BMD_{10} is 70 mg/kg bw/d. As according to the strategy paper (Apel et al., 2020), the preferred option for determining a POD is to use a benchmark dose (BMD) approach, this latter approach was followed.

6.2 Calculation of HBM-GVs

An HBM guidance value can be derived from a TRV as described in (Apel et al. 2020) using the urinary mass balance approach if specific biomarkers are excreted in the urine. The general calculation based on a single specific major metabolite is shown as (Equation 1), on several specific metabolites detected in the urine is shown as (Equation 2).

A value of 0.54 mg/kg bw/d based on the $BMDL_{10}$ related to effects on the retina and the default assessment factor of 100 was used as TRV. Data are available from human studies that show which specific metabolites are formed after DEHTP ingestion and are detectable in urine. In a study by (Lessmann et al. 2016), three male volunteers were exposed to a single oral dose of 50 mg DEHTP. 16.1% of the administered dose was recovered in the urine as specific metabolites as described in section 5.1.1. The 16.1% is made up of 13% 5cx-MEPTP, 1.8% 5OH-MEHTP, 1% 5oxo-MEHTP and 0.3% 2cx-MMHTP. The resulting urinary excretion factors (F_{ue}) are the decimals of the percentages resulting in values of 0.13 for the metabolite 5cx-MEPTP and 0.018 for 5OH-MEHTP as inserted into the formula. Since the major metabolite TPA is not a specific metabolite, it is not considered further (Weng et al. 2022; Lessmann et al. 2016). The molecular weight of the parent compound DEHTP is 390.56 g/mol (see section 3.1). The daily

urinary flow rate which is currently used as standard is 35 mL/kg bw/d for women[15] and 30 mL/kg bw/d for children and men. These values were inserted into Equation 3 for the major specific metabolite 5cx-MEPTP, into Equation 4 for 5OH-MEHTP and into Equation 5 for the sum of these two specific metabolites, which were found in the urine of the volunteers.

Equation 1: Urinary mass balance approach with one metabolite

$$HBM - GV = TRV \times \frac{\left(\frac{MW(Metabolite) \times Fue(Metabolite)}{MW(Substance)}\right)}{Daily\ urinary\ flow\ rate}$$

Equation 2: Urinary mass balance approach with two metabolites

$$HBM - GV = TRV \times \frac{\left(\frac{MW(Met.1) \times Fue(Met1) + MW(Met.2) \times Fue(Met.2)}{MW(Substance)}\right)}{Daily\ urinary\ flow\ rate}$$

Equation 3: Urinary mass balance approach for the major specific metabolite 5cx-MEPTP, here for women:

$$HBM - GV = \frac{0.54\frac{mg}{kg}bw}{d} \times \frac{\left(\frac{308.32\frac{g}{mol} \times 0.13}{390.56\frac{g}{mol}}\right)}{\frac{0.035\frac{L}{kg}bw}{d}} = 1.58\ mg/L$$

Equation 4: Urinary mass balance approach for the specific metabolite 5OH-MEHTP, here for women:

$$HBM - GV = \frac{0.54\frac{mg}{kg}bw}{d} \times \frac{\left(\frac{294.33\frac{g}{mol} \times 0.018}{390.56\frac{g}{mol}}\right)}{\frac{0.035\frac{L}{kg}bw}{d}} = 0.209\ mg/L$$

Equation 5: Urinary mass balance approach for the two metabolites 5cx-MEPTP and 5OH-MEHTP, here for women:

$$HBM - GV = \frac{0.54\ \frac{mg}{kg}bw}{d} \times \frac{\left(\frac{308.32g}{mol} \times 0.13 + \frac{294.33g}{mol} \times 0.018}{390.56\frac{g}{mol}}\right)}{\frac{0.035\frac{L}{kg}bw}{d}} = 1.79\ mg/L$$

Resulting HBM-GVs for women are 1.58 mg/L urine for 5cx-MEPTP, 0.209 mg/L urine for 5OH-MEHTP and 1.79 mg/L urine for the sum of the two specific metabolites 5cx-MEPTP and 5OH-MEHTP.

For children and men, the HBM-GVs are 1.85 mg/L, taking into account the major specific metabolite 5cx-MEPTP, 0.244 mg/L for the specific metabolite 5OH-MEHTP and 2.09 mg/L for both metabolites.

[15] Amended for women amd men according to the statement of the German HBM Commission (2025, submitted)

The derived HBM-GVs represent the concentration of the metabolite(s) in human urine up to which – for single substance evaluation – a risk for adverse health effects is not anticipated.

However, when assessing HBM results, it must be taken into account that exposure to mixtures of substances is usually present, so that the additional use of a MAF (mixture assessment factor/mixture allocation factor) or another suitable methodology should be considered for further assessments. At least structurally related substances or substances that display the same effect targets should be considered together for the next review and refinement of the HBM-GV.

6.3 Level of confidence attributed to the derived HBM-GVs

As mentioned in the HBM4EU concept document to derive HBM-GVs, it is suggested to attribute a level of confidence (low, medium or high) to each calculated HBM-GV. The level of confidence should reflect the uncertainties identified during the elaboration of the value and could constitute an adequate incentive to later revise values with an estimated 'lower' level of confidence.

The toxicological reference value of 0.54 mg/kg bw/d is based on the $BMDL_{10}$ of 54 mg/kg bw/d. In the literature search, the GLP compliant chronic toxicity/carcinogenicity study in rats by (Deyo 2008) was considered as the key study. The study was performed according to EPA OPPTS 870.4200 with 50 rats/sex/dose. Nevertheless, the key study for the derivation of the TRV is based on animal data. However, the best case for deriving HBM-GVs would have been availability of human data. Also, the data base for endocrine disruption in open literature indicate some effects, but is currently not robust enough for TRV selection. The level of confidence is therefore **medium**.

The data used for calculation of the urinary excretion fraction was derived from a study with three adult male volunteers, who received a single oral DEHTP dose of 50 mg, resulting in individual dosages between 0.55 and 0.59 mg/kg bw (Lessmann et al. 2016). As toxicokinetics are subject to considerable individual variability such as age, gender or ethnicity, it is difficult to draw general conclusions from these three individual measurements to the general population. However, since the TRV used of 0.54 mg/kg bw/d already includes a standard safety factor of 10 to cover intraspecies variability similar to a TDI, it can be concluded that the variability that has occurred is already covered to some extent but DEHTP specificities are not taken into account due to lack of data. Therefore, the level of confidence is **medium**.

According to (Apel et al. 2020), the urinary mass balance approach is applicable for compounds mainly eliminated through urine. For DEHTP, 16.1 % of the administered dose was recovered as specific metabolites in the urine. Terephthalic acid (TPA) as a non-specific metabolite of DEHTP was not quantified, but it was assumed to be the main metabolite in urine (Lessmann et al. 2016). It could be shown in an animal study with CD rats that the administered dose is almost completely excreted via urine and faeces (56.5 ± 12.1 % via faeces, 31.9 ± 10.9 % via urine and 3.6 ± 0.9 % in expelled air with 1.4 ± 0.6 % remaining in the body within 144 hours) (OECD SIDS 2003; Risk Science Center 2018). Therefore, it can be assumed that bioaccumulation is very limited and that the biomarker concentration in urine can be considered directly proportional to the amount absorbed.

Additionally, the dose, which the three volunteers received in (Lessmann et al. 2016) consisted of unlabelled DEHTP. Hence, it cannot be clearly distinguished between the exposure in this study and the regular background exposure. Nevertheless, quantification was attempted to be achieved by comparing pre-dose samples with post-dose samples where the post dose metabolite levels were several orders of magnitude higher than levels observed in the pre-dose samples as described in section Toxicokinetics and metabolism.

The **overall level of confidence** of HBM-GVs derived here is considered to be **medium**. This is mainly due to the limited availability of toxicokinetic data, as only one study with three adult male and healthy volunteers was available for the determination of urine concentrations. However, the values may be dependent on gender, age, or ethnicity, as already described. Another major reason is the lack of further human toxicological data for the derivation of the TRV.

It should be noted, though, that the levels of confidence suggested in this document are based on expert judgement regarding the accuracy of the data and the process of calculation used to derive the HBM-GVs, rather than being established in accordance with a recognized and accepted methodology.

7 References[16]

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[16] Please note that in the tables in the appendix only those primary studies are cited for which the primary source was available. Where only a secondary source was available, as indicated in the 'cited in' column, only that source is cited, and the references refer to the citation in the secondary source.

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8 Appendix – Toxicological profile

8.1 Search terms

Scopus

TITLE-ABS-KEY ((dehtp OR deht OR dotp OR {Bis(2-ethylhexyl) terephthalate} OR {6422-86-2} OR {Di(2-ethylhexyl) terephthalate} OR {Benzol-1,4-dicarbonsäure, bis(2-ethylhexyl) ester} OR {Dioctyl terephthalate} OR MEHTP OR {Mono-(2-ethylhexyl) terephthalate} OR {Benzol-1,4-dicarbonsäure, mono(2ethyl-hexyl) ester} OR {Mono-(2-ethylhexyl)-terephthalate} OR {Mono-(2-ethylhexyl)tere phthalate} OR {Mono-(2-ethylhexyl)tere-phthalate} OR {Mono(2-ethylhexyl) terephthalate} OR {Mono(2-ethylhexyl)-terephthalate} OR {Mono(2-ethylhexyl)tere phthalate} OR {Mono(2-ethylhexyl)tere-phthalate} OR {5OH-MEHTP} OR {Mono-(2-ethyl-5-hydroxyhexyl) terephthalate} OR {Benzol-1,4-dicarbonsäure, mono(2ethyl-5-hydroxyhexyl) ester} OR {Mono-(2-ethyl-5-hydroxyhexyl)-terephthalate} OR {Mono-(2-ethyl-5-hydroxyhexyl)tere phthalate} OR {Mono-(2-ethyl-5-hydroxyhexyl)tere-phthalate} OR {Mono(2-ethyl-5-hydroxyhexyl) terephthalate} OR {Mono(2-ethyl-5-hydroxyhexyl)-terephthalate} OR {Mono(2-ethyl-5-hydroxyhexyl)tere phthalate} OR {Mono(2-ethyl-5-hydroxyhexyl)tere-phthalate} OR {5oxo-MEPTP} OR {Mono-(2-ethyl-5-oxo-hexyl) terephthalate} OR {Benzol-1,4-dicarbonsäure, mono(2-ethyl-5-oxo-hexyl) ester} OR {Mono-(2-ethyl-5-oxo-hexyl)-terephthalate} OR {Mono-(2-ethyl-5-oxo-hexyl)tere phthalate} OR {Mono-(2-ethyl-5-oxo-hexyl)tere-phthalate} OR {Mono(2-ethyl-5-oxo-hexyl) terephthalate} OR {Mono(2-ethyl-5-oxo-hexyl)-terephthalate} OR {Mono(2-ethyl-5-oxo-hexyl)tere phthalate} OR {Mono(2-ethyl-5-oxo-hexyl)tere-phthalate} OR {5cx-MEHTP} OR {Mono-(2-ethyl-5-carboxylpentyl) terephthalate} OR {Benzol-1,4-dicarbonsäure, mono(2-ethyl-5-carboxylpentyl) ester} OR {Mono-(2-ethyl-5-carboxylpentyl)-terephthalate} OR {Mono-(2-ethyl-5-carboxylpentyl)tere phthalate} OR {Mono-(2-ethyl-5-carboxylpentyl)tere-phthalate} OR {Mono(2-ethyl-5-carboxylpentyl) terephthalate} OR {Mono(2-ethyl-5-carboxylpentyl)-terephthalate} OR {Mono(2-ethyl-5-carboxylpentyl)tere phthalate} OR {Mono(2-ethyl-5-carboxylpentyl)tere-phthalate} OR {2cx-MMHTP} OR {Mono-(2-carboxylmethyl-hexyl) terephthalate} OR {Benzol-1,4-dicarbonsäure, mono(2-carboxylmethyl-hexyl) ester} OR {Mono-(2-carboxylmethyl-hexyl)-terephthalate} OR {Mono-(2-carboxylmethyl-hexyl)tere phthalate} OR {Mono-(2-carboxylmethyl-hexyl)tere-phthalate} OR {Mono(2-carboxylmethyl-hexyl) terephthalate} OR {Mono(2-carboxylmethyl-hexyl)-terephthalate} OR {Mono(2-carboxylmethyl-hexyl)tere phthalate} OR {Mono(2-carboxylmethyl-hexyl)tere-phthalate}) AND (health OR adverse OR {mode of action} OR toxic* OR neurotox* OR epidem* OR cohort OR genotox* OR genetic* OR carc* OR cancer OR reproduc* OR reprotox* OR developmental OR fertil* OR disease OR neurologic* OR metabol* OR adme OR pbpk OR pbtk OR excret* OR {in vivo} OR {in vitro} OR {in silico} OR endocrine OR {half-life} OR immun* OR moa))

Pubmed

(DEHTP[Title/Abstract] OR DEHT[Title/Abstract] OR DOTP[Title/Abstract] OR "Bis(2-ethylhexyl) terephthalate"[Title/Abstract] OR "6422-86-2"[Title/Abstract] OR "Di(2-ethylhexyl) terephthalate"[Title/Abstract] OR "Benzol-1,4-dicarbonsäure, bis(2-ethylhexyl) ester"[Title/Abstract] OR "Dioctylterephthalate"[Title/Abstract] OR "Dioctyltere phthalate"[Title/Abstract] OR MEHTP[Title/Abstract] OR "Mono-(2-ethylhexyl) terephthalate"[Title/Abstract] OR "Benzol-1,4-dicarbonsäure, mono(2ethyl-hexyl) ester"[Title/Abstract] OR "Mono-(2-ethylhexyl)-terephthalate"[Title/Abstract] OR "Mono-(2-ethylhexyl)tere phthalate"[Title/Abstract] OR "Mono-(2-ethylhexyl)tere-phthalate"[Title/Abstract] OR "Mono(2-ethylhexyl) terephthalate"[Title/Abstract] OR "Mono(2-ethylhexyl)-terephthalate"[Title/Abstract] OR "Mono(2-ethylhexyl)tere phthalate"[Title/Abstract] OR "Mono(2-ethylhexyl)tere-phthalate"[Title/Abstract] OR 5OH-MEHTP[Title/Abstract] OR "Mono-(2-ethyl-5-hydroxyhexyl) terephthalate"[Title/Abstract] OR "Benzol-1,4-dicarbonsäure, mono(2ethyl-5-hydroxyhexyl) ester"[Title/Abstract] OR "Mono-(2-ethyl-5-hydroxyhexyl)-terephthalate"[Title/Abstract] OR "Mono-(2-ethyl-5-hydroxyhexyl)tere phthalate"[Title/Abstract] OR "Mono-(2-ethyl-5-hydroxyhexyl)tere-phthalate"[Title/Abstract] OR "Mono(2-ethyl-5-hydroxyhexyl) terephthalate"[Title/Abstract] OR "Mono(2-ethyl-5-hydroxyhexyl)-terephthalate"[Title/Abstract] OR "Mono(2-ethyl-5-hydroxyhexyl)tere phthalate"[Title/Abstract] OR "Mono(2-ethyl-5-hydroxyhexyl)tere-phthalate"[Title/Abstract] OR 5oxo-MEPTP[Title/Abstract] OR "Mono-(2-ethyl-5-oxo-hexyl) terephthalate"[Title/Abstract] OR "Benzol-1,4-dicarbonsäure, mono(2-ethyl-5-oxo-hexyl) ester"[Title/Abstract]

OR "Mono-(2-ethyl-5-oxo-hexyl)-terephthalate"[Title/Abstract] OR "Mono-(2-ethyl-5-oxo-hexyl)tere phthalate"[Title/Abstract] OR "Mono-(2-ethyl-5-oxo-hexyl)tere-phthalate"[Title/Abstract] OR "Mono(2-ethyl-5-oxo-hexyl) terephthalate"[Title/Abstract] OR "Mono(2-ethyl-5-oxo-hexyl)-terephthalate"[Title/Abstract] OR "Mono(2-ethyl-5-oxo-hexyl)tere phthalate"[Title/Abstract] OR "Mono(2-ethyl-5-oxo-hexyl)tere-phthalate"[Title/Abstract] OR 5cx-MEHTP[Title/Abstract] OR "Mono-(2-ethyl-5-carboxylpentyl) terephthalate"[Title/Abstract] OR "Benzol-1,4-dicarbonsäure, mono(2-ethyl-5-carboxylpentyl) ester"[Title/Abstract] OR "Mono-(2-ethyl-5-carboxylpentyl)-terephthalate"[Title/Abstract] OR "Mono-(2-ethyl-5-carboxylpentyl)tere phthalate"[Title/Abstract] OR "Mono-(2-ethyl-5-carboxylpentyl)tere-phthalate"[Title/Abstract] OR "Mono(2-ethyl-5-carboxylpentyl) terephthalate"[Title/Abstract] OR "Mono(2-ethyl-5-carboxylpentyl)-terephthalate"[Title/Abstract] OR "Mono(2-ethyl-5-carboxylpentyl)tere phthalate"[Title/Abstract] OR "Mono(2-ethyl-5-carboxylpentyl)tere-phthalate"[Title/Abstract] OR 2cx-MMHTP[Title/Abstract] OR "Mono-(2-carboxylmethyl-hexyl) terephthalate"[Title/Abstract] OR "Benzol-1,4-dicarbonsäure, mono(2-carboxylmethyl-hexyl) ester"[Title/Abstract] OR "Mono-(2-carboxylmethyl-hexyl)-terephthalate"[Title/Abstract] OR "Mono-(2-carboxylmethyl-hexyl)tere phthalate"[Title/Abstract] OR "Mono-(2-carboxylmethyl-hexyl)tere-phthalate"[Title/Abstract] OR "Mono(2-carboxylmethyl-hexyl) terephthalate"[Title/Abstract] OR "Mono(2-carboxylmethyl-hexyl)-terephthalate"[Title/Abstract] OR "Mono(2-carboxylmethyl-hexyl)tere phthalate"[Title/Abstract] OR "Mono(2-carboxylmethyl-hexyl)tere-phthalate"[Title/Abstract]) AND (health[Title/Abstract] OR adverse[Title/Abstract] OR "mode of action"[Title/Abstract] OR toxic*[Title/Abstract] OR neurotox*[Title/Abstract] OR epidem*[Title/Abstract] OR cohort[Title/Abstract] OR genotox*[Title/Abstract] OR genetic*[Title/Abstract] OR carc*[Title/Abstract] OR cancer[Title/Abstract] OR reproduc*[Title/Abstract] OR reprotox*[Title/Abstract] OR developmental[Title/Abstract] OR fertil*[Title/Abstract] OR disease[Title/Abstract] OR neurologic*[Title/Abstract] OR metabol*[Title/Abstract] OR ADME[Title/Abstract] OR PBPK[Title/Abstract] OR PBTK[Title/Abstract] OR excret*[Title/Abstract] OR "in vivo"[Title/Abstract] OR "in vitro"[Title/Abstract] OR "in silico"[Title/Abstract] OR endocrine[Title/Abstract] OR "half-life"[Title/Abstract] OR immun*[Title/Abstract] OR MOA[Title/Abstract])

8.2 Findings in humans

Table V.18: Overview of toxicokinetics and metabolism in humans

Reference	Named in	Findings
(NRW, LANUV, 2018)	-	• Biomonitoring study in human urine of 251 German children 2-6 years • 5OH-MEHTP was detected in 97% of samples (7.82-532.00 µg/L, P95 = 8.71 µg/L) • 5oxo-MEHTP was detected in 95% of samples (6.05-408.00 µg/L, P95 = 8.59 µg/L) • 5cx-MEPTP was detected in 100% of samples (86.33-5970.00 µg/L, P95 = 99.20 µg/L) • 2cx-MMHTP was detected in 59% of samples (2.21-178.00 µg/L, P95 = 2.40 µg/L)
Lessmann *et al.* 2016a	(Risk Science Center 2018)	• Urine samples from 34 male and female volunteers (aged 25–61) not known to be occupationally exposed to DEHTP were drawn • 1-mono-(2-ethyl-5-carboxyl-pentyl) benzene-1,4-dicarboxylate (5cx-MEPTP) was detected above the limit of quantification in 94% of the samples (median: 0.9 µg/L, maximum: 38.7 µg/L).
(Lessmann et al. 2016)	(Risk Science Center 2018)	• Investigation of metabolism and renal excretion in three adult volunteers administered a nominal dose of 50 mg DEHTP (actual dose 0.55-0.59 mg/kg bw) • Urine samples were collected for 48 h from directly after dosing and analysed for metabolites unique to DEHTP (terephthalic acid was not considered): 1-mono-(2-ethyl-5-hydroxy-hexyl) benzene-1,4-dicarboxylate (5OH-MEHTP), 1-mono-(2-ethyl-5-oxo-hexyl) benzene-1,4-dicarboxylate (5oxo-MEHTP), 1-mono-(2-ethyl-5-carboxyl-pentyl) benzene-1,4-dicarboxylate (5cx-MEPTP), and 1-mono-(2-carboxyl-methyl-hexyl) benzene-1,4-dicarboxylate (2cx-MMHTP). • t_{max} of 4.2 to 5.2 h was identified • Elimination half-lives for metabolites were 6.9 to 7.0 h • Percentage of urinary excretion were: 12.95% (7.0-20.4 %) for 5cx-MEPTP; 1.82% (1.3-2.4 %) for 5OH-MEHTP; 1.01% (0.6-1.6 %) for 5oxo-MEHTP; 0.28% (0.2-0.4 %) for 2cx-MMHTP • Approximately 16.1% of the dose was recovered in the urine within 48 hours as the specific metabolites analysed above, with the majority (95%) excreted within the first 24 hours

Reference	Named in	Findings
		• Carboxy metabolites are almost completely excreted in their free form (>90%), while glucuronidation is favoured for 5OH-MEHTP and 5oxo-MEHTP (>70%) • Sex or age specific differences were not investigated
Silva et al. 2015 **Silva et al. 2017**	(Risk Science Center 2018)	• Biomonitoring studies in human urine (no further information given) • Monoester mono-2-ethylhydroxyhexyl terephthalate (MEHHTP) and the oxidative metabolite mono-2-ethyl-5-carboxypentyl terephthalate (MECPTP) were detected in human urine
(Alves et al. 2017, information from the abstract)		• In a Norwegian cohort, LC/ESI-Orbitrap-MS was used to search for metabolites of DEHTP in urine and fingernails after a single oral administration of 50 mg DEHTP to three healthy male volunteers (32-45 years; body weight 85-95 kg) • Resulting doses were 0.55-0.59 mg/kg body weight and are comparable to studies on the human metabolism of phthalates and alternative plasticisers • The subjects had no occupational exposure to DEHTP and provided urine samples immediately prior to oral dosing (t=0) and collected their whole urine samples consecutively over the following 48 hours • Two isomeric forms of the DEHTP primary metabolite detected to a high degree in urine (97 % DF) and in fingernails • Sample volume was determined as the mass difference between an empty and a filled sample container

Table V.19: Overview of effects on skin and mucous membranes in humans

Reference	Named in	Findings
(Lockhart 2001a)	(Risk Science Center 2018; ANSES 2016; OECD 2003)	• 9 male and 9 female subjects were exposed to 0.01, 0.05, 0.1, 0.2, 0.5 % DEHTP in acetone as dermal application under semi-occluded conditions for 24 h with a total of three 24 h applications on days 1, 3, 5 • Score 24 hours after removal of the patches and before reapplication • Very little irritation at any of the tested concentrations • Minimal irritation with overall irritation scores from 0.00 to 0.11 with no concentration dependency
(David, Lockhart, and Ruble 2003)	(Risk Science Center 2018)	• 203 male and female volunteers aged 18 to 81 years were tested for skin irritation and sensitisation after DEHTP exposure • Subjects were treated with 0.5% (v/v) DEHTP under a semi-occlusive patch for 24 hours three times a week for three weeks and challenged at a naïve site after a 2-week rest period • Only one subject showed irritation in the form of mild erythema, which occurred in at least 4 out of 9 exposures with the same subject showing delayed reaction to DEHTP exposure • The authors of the study concluded that the substance is not irritating and not sensitizing to humans
(Lockhart 2001b)		• Skin sensitisation study using the Human Repeat Insult Patch Test (HRIPT) was conducted with 18 volunteers (9 female, 9 male) • Subjects were exposed to 0.5% DEHTP in acetone for nine repeated dermal applications under semi-occlusive conditions over a three-week induction period • Challenge with 0.5% applied to the skin after two weeks • DEHTP appeared to be non-irritating and non-sensitising in all subjects
Lockhart 2001c	(UNEP 2003 as cited in (ToxServices 2012; OECD 2003)	• Skin sensitisation (modified Draize procedure)was investigated in 44 male and 197 female subjects • Induction phase was three times a week for three weeks with 0.2 ml of 0.5 % DEHTP followed by 10-17 days rest period with a single challenge afterwards • DEHT was non-irritating and non-sensitizing in all volunteers since adverse effects were not attributed to the test substance

Table V.20: Overview of carcinogenic effects in humans

Reference	Findings
(Lee et al. 2020)	• Case-control study in women of childbearing potential to investigate the association between DEHTP exposure and development of uterine fibroids, benign tumours of the uterus that develop from smooth muscle tissue • Study included 102 women from Korea, with 32 cases and 70 controls based on gynaecological examinations from 2015 to 2016 • Mean age was 38 years (cases: 38.2 ± 5.3; controls: 37.8 ± 5.1), the mean BMI was 22.7 ± 2.7 kg/m^2 for the cases and 22.8 ± 4.0 kg/m^2 for the controls • Confounders such as age, income, alcohol consumption and BMI were comparable in both groups, but no information was given on the participants' smoking behaviour • Participants' urine was analysed for the corresponding metabolites • Odds ratio for uterine fibroids was significantly increased with DEHTP exposure, among other things; the metabolite OH-MEHTP was detected in 40.6 % of cases, compared to 29.1 % in the controls • Authors address various weaknesses of the study, including the small group size and the fact that the half-life of various metabolites is too short to be adequately quantified • Further studies are recommended by authors
(Cho et al. 2021)	• Connection between leiomyomas (oestrogen-dependent benign tumours of unknown origin) of the uterus and DEHTP was investigated • Patients who had undergone laparoscopic surgery between March 2017 and February 2019 were included • Occupational exposure to reproductive toxicants, hormone therapy, malignancy or other reproductive treatment within the previous 3 months were exclusion criteria 97 patients included, including those with leiomyoma (n = 45), leiomyoma with endometriosis (n = 29), leiomyoma with adenomyosis (n = 10) and leiomyoma with endometriosis and adenomyosis (n = 13) • 30 patients were randomly selected from whom samples of both leiomyomas and adjacent normal myometrial tissue were taken • Urine concentrations of 20 phthalate metabolites and seven metabolites of DEHTP were measured by UHPLC-MS/MS • 12 phthalate metabolites and 3 DEHTP metabolites 5cx-(MEHTP, 5OH-MEHTP, and 5oxo-MEHTP) were detected in 70 % of the study participants • Gene sequences ESR1 and PGR were reduced in patients with leiomyoma • 5oxo-MEHTP showed positive association with methylation of oestrogen receptor ESR1 in leiomyoma and therefore behaved differently to metabolites of other phthalates • Authors name limitations of the study such as small number of test subjects with large differences in diseases, as some participants also suffered from endometriosis and adenomyosis and are in favour of a repetition

Table V.21: Overview of effects on fertility in humans

Reference	Findings
(Long et al. 2021)	• Cross-sectional study with women > 20 years of age not being pregnant or breastfeeding and had not undergone oophorectomy (n = 698 premenopausal, n = 557 postmenopausal) to investigate an association between urinary phthalate metabolites, serum sex hormones and relevant covariates • Study showed that premenopausal women had higher DEHTP concentrations than postmenopausal women • DEHTP was not statistically significantly associated with altered testosterone or oestradiol levels but was associated with a higher testosterone to oestradiol ratio in postmenopausal women • According to the authors, these results provide initial evidence that newer alternatives like DEHTP may also be hormonally active, similar to the older generation of plasticisers but postmenopausal women in the study had lower exposure levels to DEHP, DINCH, and DEHTP compared to premenopausal women • Further research is needed
(Pacyga et al. 2023; 2021)	• Pregnant women aged 18 - 40 included in study during early pregnancy to investigate the relationship between phthalate exposure and biomarkers of oestrogen and testosterone in urine • Sums of urine concentrations of the metabolites and of eight oestrogen and two testosterone metabolites measured at weeks 13, 28 and 34 of pregnancy were calculated • Various confounders such as age, ethnicity, education, smoking habits in the first trimester, BMI, diet, time of conception, gestational age and sex of the foetus were eliminated • A positive correlation was established between the sum of DEHTP metabolites and the sum of oestrogens (doubling of the DEHTP metabolites was accompanied by a 3.2 % increase in oestrogens) • Correlation was particularly pronounced when the foetus was female and negative in male foetuses

8.3 Animal findings

Table V.22: Overview of toxicokinetics and metabolism in animals or in vitro.

Reference	Named in	Findings
(Barber et al. 1994), **Fox et al. 1984**	(OECD 2003; Risk Science Center 2018)	• hydrolysis investigated in vitro using rat intestinal homogenate fractions from the small intestine after 16 hours of fasting • DEHTP was added and samples were taken after 0, 5, 10, 20 and 30 minutes • DEHTP was hydrolysed to 2-ethylhexanol and terephthalic acid after about 10 minutes, the rest remained unchanged without further metabolization to 2EH • half-life of the hydrolysis of DEHTP was 53.3 min • 1.97 moles of 2-ethylhexanol were formed per mole of DEHTP, which indicates complete hydrolysis to terephthalic acid according to first-order kinetics
(Barber et al. 1994), Enriquez et al. 1984	(OECD 2003; Risk Science Center 2018)	• 10 male CD(SD) COBS rats were exposed once to 100 mg/kg partly radiolabelled DEHTP via gavage • Exhaled air, urine, and feces were collected for up to 144 hours • mean overall recovery rate (± SD) for ^{14}C-labelled DEHTP was 93.0 ± 2.2 % • Most of the radioactivity was eliminated in the faeces (56.5 ± 12.1%) and urine (31.9 ± 10.9%), with smaller amounts in expired air (3.6 ± 0.9%) • 1.4 ± 0.6% of the dose remained in the carcass. Of the approximately one half of the material that was absorbed, 73% was excreted in the urine and 8.3% was completely metabolized to $^{14}CO_2$ • Amount in faeces mainly as unchanged DEHTP (36.6 ± 13.4 %), small amounts of mono(2-ethylhexyl) terephthalate (MEHTP) (2.5 %) and polar metabolites, such as the glucuronic acid conjugate of 2-ethyl-5-hydroxyhexanoic acid • Metabolites in urine included terephthalic acid (51% of the dose), oxidised metabolites of 2-ethylhexanol and MEHTP + glucuronic and sulphuric acid conjugates (approximately 10% of the dose) • 50.5% of the total dose (77% of the absorbed dose) was detected as unlabelled terephthalic acid (TPA) in the urine • 3.6 ± 0.9% of the dose was measured in the breath • Less than 2 % of the administered radioactivity was found in the animal body • With the exhaled fraction, an average of 90.7% of the dose was thus detected either as unchanged DEHTP in the faeces, unlabelled terephthalic acid in the urine or as^{14} CO_2 in the exhaled air

Reference	Named in	Findings
		• Almost one-third of the radioactivity in the urine (10 % of the dose) was present as glucuronide and sulphate conjugates • 90-95 % of the total activity in the feces was unchanged [^{14}C]DEHT • Small amounts of ^{14}C were found in the tissues, with the highest amounts detectable in the liver (4.5-fold) and adipose tissue (3.5-fold) • In brain, large and small intestine, heart, lungs, kidneys, spleen, testes the level was lower than in the animal body as a whole • authors conclude from their data that MEHTP and its metabolites are formed at a maximum of 9.3 % of the orally administered DEHTP dose • radioactivity contained in the urine was 95% excreted within 24 hours, 99% of the radioactivity contained in the faeces was excreted within 48 hours • 75% of the radioactivity found in body fat was extractable in acetone, whereas only 10% of the radioactivity found in the liver was extractable
Guerin et al. 2002	(OECD 2003; Risk Science Center 2018)	• In vitro skin absorption (OECD Guideline Document for the Conduct of Skin Absorption Studies, No. 28 + OECD 428) • mean absorption rate of DEHTP was 0.103 +/- 0.053 µg/cm^2/hr for cells treated with the test substance. The corresponding permeability constant was determined to be 8.39 x 10^{-8} cm/hr • mean total test substance recovery was 103.8% +/- 6.3%. • absorption rate of DEHTP through dermatomed human skin was found to be 0.103 +/- 0.052 µg/cm^2/hr ("extremely slow" penetrant relative to other chemical species) • mean damage ratio for skin treated with DEHTP was 1.14 +/- 0.23 which is within the range of damage ratios for skin exposed to physiological saline no significant damage to skin
(Silva et al. 2015)		• metabolism of DEHTP in human liver microsomes in vitro • 200 µl of female human liver microsomes were incubated with DEHTP (769.6 µg/ml) for 5 hours • After 5 hours of incubation, terephthalic acid and mono-2-ethyl hydroxyhexyl terephthalate (MEHHTP) were identified • Other potential metabolites are MEHTP and two other oxidative metabolites MEOHTP and MECPTP being determined by their mass spectrometric fragmentation patterns • After 27 hours of incubation terephthalic acid is the main metabolite and other metabolites are MEHTP, MEOHTP, MEHHTP and MECPTP

Reference	Named in	Findings
		• DEHTP and DEHP produced similar metabolites, but their metabolite profiles differed significantly
unnamed study report 1984	(ECHA 2023)	• ECHA reports eight metabolites determined in urine of CD(SD) COBS rats being gavaged with a single dose of 100 mg/kg using HPLC: Terephthalic acid, mono-(2-ethyl-4-carboxypropyl) terephthalate, mono-(2-(carboxymethyl)-hexyl) tere-phthalate, 2-ethylhexanol, 2-ethylhexanoic acid, 5-hydroxy-2-ethylhexanoic acid, 5-hydroxy-2-ethylhexanlactone and 2-heptanone • mean total recovery of ^{14}C was 93.0 ± 2.2%. Most of the radioactivity was eliminated in the feces (56.5 ± 12.1%) and urine (31.9 ± 10.9%), with smaller amounts in expired air (3.6 ± 0.9%). Approximately 1.4 ± 0.6% of the dose remained in the carcass • major excretory products are TPA and di (2-ethylhexyl) terephthalate, together accounting for 87.1% of the dose
(Kademoglou et al. 2018)		• bioavailability investigated in vitro using two different artificial lung fluids: artificial lysosomal fluid (ALF, pH 4.5), to obtain intracellular acidic lung fluid after particle contact and phagocytosis by alveolar macrophages, and Gamble's solution (pH 7.4), a surrogate for extracellular fluid to study deep lung deposits • 0.2 g of the respective dust was mixed with 20 ml of each lung fluid (volume of an adult healthy non-smoker) • inhaled bioavailability decreased with increasing molecular weight and LogP of the phthalate esters analysed • bioavailability very low in the test: Only 4.9 % detected in Gamble's solution, 4.6 % in ALF
(McNally et al. 2023)		• PBPK model for DEHTP from existing in silico, in vitro and human data • intrinsic hepatic clearance scaled from in vitro to in vivo, the unbound plasma fraction and partition coefficients were determined algorithmically • model was used to determine the metabolism and kinetics of a single oral dose of 50 mg • predictions of the model and already calculated model parameters deviated considerably from measured values due to the highly lipophilic properties of the chemical class

Table V.23: Overview of acute toxicity studies

Reference	Named in	Route of exposure	Species	Results (LD50/LC50)
Gordon 1975, Terhaar 1975b	(OECD 2003) (Risk Science Center 2018)	Oral	Rat	>3,200 mg/kg bw
Gordon 1975, Terhaar 1975b	(OECD 2003) (Risk Science Center 2018) (Greenscreen Assessment/UNEP 2003 report)	Oral	Mouse	>3,200 mg/kg bw
Shepard 1994	(OECD 2003) (Greenscreen Assessment/UNEP 2003 report) (Greenscreen Assessment)	Oral	Rat	>5,000 mg/kg bw
Timofiyevskaya, 1982	(Risk Science Center 2018)	Oral	Mouse	No LD50 up to 20,000 mg/kg bw, but mortality observed with preceding clinical signs of excitation followed by CNS inhibition
Gordon 1975, Terhaar 1975b	(UNEP 2003 as cited in (ToxServices 2012; OECD 2003; Risk Science Center 2018)	Dermal	Guinea pig	>19,670 mg/kg bw
Gordon 1975, Terhaar 1975b	(OECD 2003)	i.p.	Rat	>3,200 mg/kg bw
Gordon 1975, Terhaar 1975b	(OECD 2003)	i.p.	Mouse	>3,200 mg/kg bw

Table V.24: Overview of studies with repeated exposure in animals.

Reference	Named in	Species	Dosage	Administration, duration	NOAEL/ LOAEL	Findings
(Deyo 2008)	(Risk Science Center 2018; CHAP 2014; ToxServices 2012)	Fischer 344 rats (50/sex/dose)	0, 1500, 6000, 12000 ppm 0, 79, 324, 666 mg/kg bw/d, males 0, 102, 418, 901 mg/kg bw/d, females	Diet for 104 weeks	NOAEL: 79 mg/kg bw/d (males), 102 mg/kg bw/ d (females)	• No increased mortality • Reduced bw gain in MD and HD. Body weight gain of males and females receiving 12,000 ppm was statistically significantly lower than controls at all intervals (0–16, 0–52 and 0–104 weeks). The body weight gain of males and females receiving 6000 ppm was statistically significantly lower than controls during the first year of the study (weeks 0–52), at the end of the study, the overall body weight gain of animals receiving 6000 ppm was similar to that of the controls. DEHTP had no effect on body weight or weight gain at 1500 ppm at any time. • Haematology, blood chemistry, and urinalysis were without findings • DEHTP increased the relative liver weights in females (sig. at 901 mg/kg bw/d) and males (not sig. at 666 mg/kg bw/d) • Absolute kidney weights were sig. decreased in high and mid dose males and females, but were histologically without any significant increases in kidney lesions • the incidence of portal lymphoid foci was increased (M: 666 mg/kg bw/d) • Histopathologically, sign. increased incidence of unilaterally small epididymides was observed in animals that died or were killed prematurely • Dark discoloured kidneys and cloudy eyes were observed in high dose females

Reference	Named in	Species	Dosage	Administration, duration	NOAEL/ LOAEL	Findings
(Barber and Topping 1995)	(OECD 2003; ANSES 2016; CHAP 2014; ToxServices 2012; Risk Science Center 2018)	SD rats (20/sex/group) (no limitations)	0, 0.1, 0.5, 1.0 % (0, 54, 277, and 561 mg/kg bw/d for male rats, 0, 61, 309, and 617 mg/kg bw/d for female rats)	Diet, 90 days	NOAEL: 0.5 % (277/309 mg/kg bw/d)	• No increased mortality; Feed intake, bw gain unaffected • Increase in absolute (not sign.) and relative (sign.) liver weight at 1% • Haemoglobin, haematocrit, MCV and MCH were slightly increased in HD males, MCH also in mid dose, in females MCV and MCH slightly but significantly increased in MD and HD • Clinical chemistry was unremarkable • No treatment-related effects at necropsy or histopathological examination
Terhaar 1975	(ANSES 2016; Risk Science Center 2018)	Sprague-Dawley rats (5 males/group)	0, 0.1 1 % (0, 85, 885 mg/kg bw/d)	Diet, 14 days, 5 d/week	NOEL: 885 mg/kg bw/d (1.0 %)	• No mortality observed • Effects on clinical parameters were within historical controls • Tracheitis and bronchiolitis were observed in high dose group, but considered not relevant due to same effects in control group
Topping et al. 1987, Eastman Kodak Co. (1975)	(OECD 2003; ANSES 2016; Risk Science Center 2018; CHAP 2014)	F 344 rats (5/sex/group) (stability of test substance in feed not determined above 1 %)	0, 0.1, 0.5, 1, 1.2 or 2.5 %, (0, 86, 431, 861, 1033, 2154 mg/kg bw/d for males; 0, 98, 490, 980, 1176, 2450 mg/kg bw/d for females)	Diet, 21 days	NOAEL: 1% (861/980 mg/kg bw/d)	• Clinical abnormalities, decreased bw and food consumption, serum triglyceride levels decreased for m but increased for f in HD animals, total protein increased for f at 2.5 and 1.2 %. Relative liver weights increased in 2.5 % of both sexes and in 1.0 % and 1.2 % of females (exclusively due to reduced terminal bw), abs. kidney weight changed from 1.2 %, slightly increased hepatic peroxisomes at 2.5%

Reference	Named in	Species	Dosage	Administration, duration	NOAEL/ LOAEL	Findings
Gordon 1975, Terhaar 1975a	(OECD 2003; ANSES 2016; Risk Science Center 2018)	Dunkin-Hartley guinea pigs (5 animals) (sex not specified, insufficient number)	0.5 ml (813 – 1144 mg/kg bw/d)	Dermal, 10 days	LOAEL: 0.5 ml	• No mortality or signs of skin absorption or systemic toxicity noted • Moderate erythema in one animal and severe erythema in the other four. Slight oedema was observed for all animals. The reaction did not change over the course of two weeks. Necrosis and eschar were not observed
Gordon 1986, Eastman Kodak Co. 1983, (Barber 1994)	(OECD 2003; Risk Science Center 2018)	SD rats (5/group) (only one sex, unclear test atmosphere generation)	0, 46.3 mg/m^3	Inhalation for 2 weeks (6 h/day, 5 days/week)	NOAEL: 46.3 mg/m^3	• No signs of toxicity observed • The study was poorly described, limiting its interpretation • No primary data available
Terhaar 1975	(ANSES 2016; Risk Science Center 2018)	Albino rats (5 males)	0.0718 mg/L	Inhalation, 14 days (6 h/day)	NOEL: 0.0718 mg/L	• No mortality occurred and only minor changes were noted, which are not further specified in (ANSES 2016)
Unnamed 1975	(ECHA 2023)	Sprague-Dawley (male)	vapor concentration range of 0.0463-0.0926 mg/L	Inhalation, 14 weeks (6 h/day, 5d/week)	NOAEL: 0.0781 mg/L	• No mortality observed; no significant treatment-related gross or microscopic effects observed at necropsy
(Wirnitzer et al. 2011)		Wistar rats (6/sex/dose)	0, 38.2, 114.5 or 381.6 mg/kg bw/d	Intravenously, 28 days	NOAEL: 381.6 mg/kg bw/d	• Study is described as being conducted in accordance with OECD guideline 407 but according to the guideline the route of exposure would be the oral route • No increased mortality, one death due to catheter malfunction • No clinical findings, bw, food intake comparable to ctrl • No haematological findings • Triglycerides sign. increased in HD females, hormone T3 increased in HD males but without

Reference	Named in	Species	Dosage	Administration, duration	NOAEL/ LOAEL	Findings
						histopathological correlates and therefore considered not treatment related • Urine analysis without findings despite of decreased pH values in HD males and females • Hypertrophy of the pulmonary arteries and perivascular granulocytes were attributed to exposure via the catheter, fat deposits in Kupffer cells to the fat-containing vehicle, as these also occurred in the vehicle control • No immunological findings • According to the authors, the HD corresponds to 1580 times the concentration to which a patient is exposed in the worst case within 24 hours when DEHTP is released from a 200 cm long infusion tube with lipid emulsions

Table V.25: Overview of studies on skin and eye irritation

Reference	Named in	Findings
Gordon 1975, Terhaar 1975b, (Eastman Kodak Co. 1975)	(UNEP 2003 as cited in (ToxServices 2012; OECD 2003; Risk Science Center 2018; ANSES 2016; Versar 2010; Ball, McLellan, and Bhat 2012)	• Undiluted DEHTP at 4920, 9840 or 19,680 mg/kg conducted in 1 male guinea pig per group under occlusive dressing for 24 hours, followed by a 14-day observation period • No erythema observed, moderate (HD) to severe (LD and MD) oedema with no dose dependency • After 2 weeks, the MD animals appeared normal, slight desquamation was observed in LD and HD • Study deviated significantly from the currently accepted guidelines (exposure should last 4h, instead of 24h, at least 3 animals/dose instead of 1, maximum dose should be 0.5g instead of 5-20 g • According to ANSES (2016) reliability is low, observed responses are likely to the overly stringent test conditions
Product Safety Laboratories 2006	(ANSES 2016; Ball, McLellan, and Bhat 2012)	• Skin irritation in GLP compliant study according to OECD 404 • 0.5 mL undiluted DEHTP applied dermally to 2 male and 1 female New Zealand white rabbits under occlusive conditions for 4 h • No erythema or oedema at any time point • DEHTP was classified as non-irritating
Gordon 1975, Terhaar 1975b, (Eastman Kodak Co. 1975)	(OECD 2003; Risk Science Center 2018), (Versar 2010; Ball, McLellan, and Bhat 2012)	• Study similar to OECD 405, acute eye irritation/corrosion was investigated with 0.1 ml of undiluted DEHTP in the eyes of 6 rabbits with 3 eyes washed after 30 seconds, 3 not washed • Slight eye irritation. Immediate washing did not alter the irritant response • At one-hour post-dose, slight erythema was observed on the palpebral conjunctiva and nictitating membranes of all animals • After 48h all animals appeared normal

Reference	Named in	Findings
Moore 2006	(ANSES 2016; Risk Science Center 2018; ToxServices 2012)	• OECD 405-compliant study in 1 male and 2 female New Zealand White rabbits • Exposure of one eye to 0.5 ml of undiluted DETP for 4 hours followed by a 72-hour observation period • No signs of corneal opacity or iritis were noted throughout the study. Conjunctivitis and redness were reported up to 48 hours after administration • Effects were fully reversible within 72 hours • DEHTP is not considered as an eye irritant
Terhaar 1975	(ANSES 2016)	• Non-GLP-compliant eye irritation/corrosion study in New Zealand White rabbits (n=6, sex not specified) • Undiluted DEHTP was applied in one eye • 24 hours after administration, one rabbit showed adnexal coloration of the nictitating membrane • 48 hours after exposure, all animals had recovered and appeared normal • Classification as an eye irritant is not justified as all effects were reversible within 48 hours

Table V.26: Overview on studies on skin sensitization

Reference	Named in	Findings
Sharp 1975a	(OECD 2003)	- 10 guinea pigs in footpad method - Induction phase: 5 animals were given a single injection of 1% test substance - Challenge phase: 1-week post-induction, challenge with topical application of 1% - No sensitizing potential observed in guinea pigs
Gordon 1975	(OECD 2003; ANSES 2016; Risk Science Center 2018)	- 5 male guinea pigs in footpad method - Induction phase: 5 animals were given a single injection of 1% test substance - Challenge phase: 1-week post-induction, challenge with topical application of 0.1% - No sensitizing potential observed in guinea pigs
Sharp 1975b, Terhaar 1975a	(OECD 2003)	- 15 guinea pigs in footpad method - Induction phase: 5 animals were given a single injection of 1% test substance in the footpad - Challenge phase: 1-week post-induction, challenge with topical application of 1% - No sensitizing potential observed in guinea pigs (study unreliable)
Eastman Kodak Co. 1975	(Risk Science Center 2018)	- DEHTP was reported to act as a sensitizer in guinea pigs, producing a strong skin sensitization reaction in 2/10 guinea pigs tested and a weak reaction in 6/10 guinea pigs (no further information given)
Gordon, 1986	(ANSES 2016; ToxServices 2012)	- Non-GLP-compliant skin sensitisation study in guinea pigs (strain/sex not specified, n = 5) - 1% DEHTP by injection into the footpad, followed by 1% dermal application challenge - No signs of sensitisation observed - (ANSES 2016) categorised this study as unreliable due to limited reporting

Table V.27: Overview of the in vitro genotoxicity studies

Reference	Named in	Findings
(Barber 1994)	(OECD 2003; ANSES 2016; ToxServices 2012; Risk Science Center 2018)	- Ames test negative at 1, 10, 100, 1000, 10,000 µg/plate (limit test) ± S9, similar to OECD 471 (1983) in Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100
(Barber 1994)		- Ames test negative with MEHT, a metabolite of DEHTP at 25 – 5000 µg/plate ± S9 in Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100
(Barber 1994), **Ivett 1984**	(OECD 2003; ANSES 2016; Risk Science Center 2018)	- In vitro chromosomal aberration test with CHO cells negative at 700, 800, 900, 1000 nl/ml ± S9, similar to OECD 473 (1983)
(Barber 1994) **Barber and Beilman 1984**	(OECD 2003; ANSES 2016; CHAP 2014; Risk Science Center 2018)	- CHO/HGPRT in vitro mammalian mutagenicity assay negative at 1.25, 2.5, 5, 10, and 20 nL/mL ± S9, similar to OECD 476 (1984)
Barber et al. 1984	(OECD 2003)	- Modified Ames test for urine analysis negative at 0.001, 0.01, 0.1, 1, 10 mg/plate ± S9 (0, 0.02, 0.06, 0.2 0.66, 2 ml urine/plate) in Salmonella typhimurium/TA1535, TA1537, TA1538, TA98, TA100, urine from male SD rats treated daily by gavage with 2000 mg/kg bw/d DEHTP for 15 days
(Divincenzo et al. 1985)	(ANSES 2016; CHAP 2014; Risk Science Center 2018)	- Supportive Ames test with negative results in Salmonella strains TA-98, TA-100, TA-1535, TA-1537 and TA-1538 ± S9 with urine from rats fed DEHTP in the diet at 2000 mg/kg bw/d for 15 days

Table V.28: Overview of carcinogenicity studies in animals

Reference	Named in	Findings
(Deyo 2008)	(Risk Science Center 2018; ANSES 2016)	- GLP-compliant 104-week carcinogenicity/chronic toxicity study according to EPA OPPTS 870.4200 - F-344 rats (50/sex/dose) were exposed to DEHTP via diet at 0, 1500, 6000 or 12000 ppm for 104 weeks, corresponding to 0, 79, 324 and 666 mg/kg bw/day for males and 0, 102, 418 and 901 mg/kg bw/day for females. - Body weights significantly changed in the high dose group compared to controls - no other statistically significant differences in the incidence of specific tumours between the treated and control groups of animals that died before the end of the study or were killed at the end of the study. Among animals surviving to study termination, the only significant increase in tumour incidence was for large granular cell lymphomas in low-dose males (13/29, 19/26, 16/26, 8/26 for control, low, intermediate and high dose groups, respectively) but without dose dependence and was therefore evaluated as not treatment related. - NOAEL for carcinogenicity is 12000 ppm, i.e., the highest dose tested.
Danish EPA 2010	(Risk Science Center 2018)	- Combined chronic toxicity and carcinogenicity study - DEHTP was administered to male and female Fischer-344 rats in diets at doses of 20, 142, and 1000 mg/kg bw/day. - No treatment-related effects were evident from the gross and histopathologic examinations conducted at 6 and 12 months. - After 18 months, hyperplasia and/or transitional cell adenomas of the urinary bladder and adenomas or adenocarcinomas of the uterus (no additional information given) - The authors of the assessment report cited the Danish EPA considering the study negative for carcinogenicity, i.e. the NOAEL was 1000 mg/kg bw/day - Due to the sparse information of the study and the assessment of the authority, the tumours in the urinary bladder are assigned only minor significance.

Table V.29: Overview of effects on fertility in animals

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Faber et al. 2007b, Stump 2001b)	(OECD 2003; ANSES 2016; Risk Science Center 2018; CHAP 2014)	Crl:CD(SD)IGS-BR rats (30/sex/group), 2-gen study according to OECD 416	0, 3000, 6000, 10000 ppm Doses received F0: 3000 ppm: 133 to 182 mg/kg/day for adult male rats, and 184 to 478 mg/kg/day 6000 ppm: 265 to 367 mg/kg/day for adult male rats, and 372 to 940 mg/kg/day for adult female rats 10000 ppm: 447 to 614 mg/kg/day for adult male rats, and 595 to 1349 mg/kg/day for adult female rats Doses received F1: 3000 ppm: 159 to 256 mg/kg/day for adult male rats, and 206 to 516 mg/kg/day for adult female rats 6000 ppm: 320 to 523 mg/kg/day for adult male rats, and 423 to 1036 mg/kg/day for adult female rats 10000 ppm: 552 to 893mg/kg/day for adult male rats, and 697 to 1549 mg/kg/day for adult female rats	Diet, minimum 70 days prior to mating. For males until euthanasia, for females until lactation, repeated for offspring F1	NOAEL for fertility: 10000 ppm NOAEL for parental and pup systemic toxicity: 3000 ppm	Three treatment related deaths for HD females of F0, seven for HD females in F1 Effects in adults Body weights F0: decreased bw in HD females throughout gestation and lactation, sporadic increases and decreases in males and females during premating without clear pattern F1: decreased bw in MD and HD males, in females without clear pattern Organ weights F0: decreased absolute pituitary gland weights in HD males and increased relative liver weights in MD and HD females F1: decreased lower mean absolute liver (-14.7%), kidney (-11.5%), spleen (-13.4%), testes (-7.9 and -10.4%) and adrenal gland (-14.6%) weights in HD males, relative weights were unaffected, spleen weights also decreased in LD and MD males, increased brain weights in HD males, decreased absolute kidney and uterus weights in HD females, increased relative brain, liver, spleen, and thymus gland weights in HD females No histopathological changes observed. Effects in pups Body weights F1: Decreased bw gain and food consumption in MD und HD pups. Decreased bw gain with decreased food consumption of dams in MD and HD animals Reproductive parameters, litter parameters, spermatogenic endpoints in F1 and F2 unaffected

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Rajkumar et al. 2022		Sertoli cell line (TM4), a male spermatogonial cell line (C18-4) and two steroidogenic cell lines (MA-10 Leydig and KGN granulosa), in vitro test	0.001-100 µM	48 hours	100 µM	No effects observed in C18-4, MA-10 and KGN up to the highest concentration. Oxidative stress in TM4 at a benchmark concentration of 28.5 µM (3.4-71.0)
Potts et al. 2025		*In vivo* studies: young adult CD-1 mice *In vitro* studies: Isolated untreated ovarian follicles	With vehicle: 10 µg/kg bw/d, 100 µg/kg bw/d, or 100 mg/kg bw/d DEHTP Vehicle, 0.1, 1, 10, or 100 µg/mL of DEHTP or MEHTP	Oral, for 10 days 24 – 96h		DEHTP exposure altered follicle counts compared to control. DEHTP exposure also decreased expression of cell cycle regulators and apoptotic factors compared to control. Follicle growth was reduced compared to controls, and expression of the cell cycle regulator Cdkn2b was increased.

Table V.30: Overview of developmental toxicity in animals

Reference	Named in	Species, study type	Dosage	Administration, duration	NOAEL / LOAEL	Findings
(Faber et al. 2007a)	(ToxServices 2012; CHAP 2014; Risk Science Center 2018)	Mated female Crl:CD(SD)IGS-BR rats (25/group), Prenatal Developmental Toxicity Study according to OECD 414	0, 0.3, 0.6, 1.0% 0, 226, 458, and 747 mg/kg bw/d	Diet, GD 0-20	NOAEL 747 mg/kg bw/d, (CHAP 2014): NOAEL of 458 mg/kg bw/d for maternal (based on mean and net maternal body and liver weight decrements) and developmental tox (variations)	Number of viable and non-viable foetuses, resorptions and implantation sites, and corpora lutea did not differ from controls. No visceral or skeletal anomalies and no signs of developmental toxicity. No effect on wet or blotted uterine weight parameters. Maternal body weight gain was reduced during GD 16–20 in the high dose group, but body weights were similar to controls during the entire treatment period. A significant increase in absolute liver weight was also reported for high dose rats. A dose-related increase in the incidence of 14th rudimentary ribs was observed at 0.6 and 1% reaching statistical significance at 1% but according to the authors this is amongst the most common skeletal developmental variations in laboratory rats which represent transient changes at a particularly labile skeletal region and have been shown not to persist throughout development and into adulthood. The authors do not consider this derivation to be admissible because the variations are not considered to be adverse.
(Faber et al. 2007a)	(CHAP 2014; Risk Science Center 2018)	Mated female Crl:CD1(ICR) mice (25/group), Prenatal Developmental Toxicity Study according to OECD 414	0, 0.1, 0.3, and 0.7%; 0, 197, 592, and 1382 mg/kg bw/d	Diet, GD0-18	Developmental NOAEL: 1382 mg/kg bw/d Maternal tox NOAEL: 197 mg/kg bw/d	Number of viable and non-viable foetuses, resorptions and implantation sites, and corpora lutea did not differ from controls. No visceral or skeletal anomalies and no signs of developmental toxicity. Signs of maternal toxicity were limited to increased liver weight in the mid and high dose group.

Reference	Named in	Species, study type	Dosage	Administration, duration	NOAEL / LOAEL	Findings
(Faber et al. 2007a)		Sexually immature female rats, uterotrophic assay for estrogenic activity	20, 200, or 2000 mg/kg bw/d	Gavage, PND 19-21	NOAEL: 2000 mg/kg bw/d	No antiandrogenic effects observed in this study
(Faber 2007b)	(OECD 2003; ANSES 2016; Risk Science Center 2018; CHAP 2014)	Crl:CD(SD)IGS-BR rats (30/sex/dose), 2-gen study according to OECD 416	0, 3000, 6000, 10000 ppm; Doses received F0: 3000 ppm: 133 to 182 mg/kg bw/d for adult male rats, and 184 to 478 mg/kg bw/d for adult female rats 6000 ppm: 265 to 367 mg/kg bw/d for adult male rats, and 372 to 940 mg/kg bw/d for adult female rats 10000 ppm: 447 to 614 mg/kg bw/d for adult male rats, and 595 to 1349 mg/kg bw/d for adult female rats Doses received F1: 3000 ppm: 159 to 256 mg/kg/ rats, 206 to 516 for adult female rats 6000 ppm: 320 to 523 mg/kg/ rats, 423 to 1036 for adult female rats 10,000 ppm: 893mg/kg/day for male and 697 to1549 for adult female rats	Diet, males: 10 weeks prior and during mating; females: 70 days prior mating until lactation; offspring starting on PND 22	NOAEL 158 mg/kg bw/d for parental systemic effects, 530 mg/kg bw/d for developmental effects	Mean litter sizes, number of pups born, percentages of males per litter at birth and postnatal survival were unaffected Female rats displayed systemic toxicity in the 516, and 860 mg/kg bw/d groups including decreased food consumption. Relative spleen and thymus weight were reduced and relative brain weight increased in various populations of rats. Absolute (F0) and relative (F0, F1) liver weights were increased in mid- and high dose females but were not correlated to morphological changes in the liver. Significant reduced body weight and feed consumption in high dose groups and mid-dose F1 dams. Developmental landmarks, balanopreputial separation delayed in F1 HD pups due to decreased growth rate. The mean day of attainment of balanopreputial separation in F1 HD males was equal to historic control value and is therefore not considered adverse.

Reference	Named in	Species, study type	Dosage	Administration, duration	NOAEL / LOAEL	Findings
Gray et al. 2000	(OECD 2003; ANSES 2016; ToxServices 2012; Risk Science Center 2018)	SD rats (8 treated + 10 ctrl), study investigated the potential for several phthalate esters to alter sexual differentiation in male rats	0, 750 mg/kg bw/d	Gavage, GD14-PND3	NOAEL for maternal tox: 750 mg/kg bw/d NOAEL for teratogenicity: 750 mg/kg bw/d	Endpoints specific to androgen disruption (reduced anogenital distance, retained nipples, cleft phallus, hypospadias, undescended testes, blind vaginal pouch, epididymal agenesis, underdeveloped accessory sex glands, and histological alterations in the testes) were investigated, no significant differences in any of the parameters determined
Knapp 2005	(ANSES 2016)	Female CD-1 mice (25/group), GLP-compliant developmental toxicity study (OECD 414)	0, 197, 592, 1382 mg/kg bw/d	Diet, GD 0-18	NOAEL for maternal tox: 197 mg/kg bw/d NOAEL for teratogenicity: 1382 mg/kg bw/d	No effects on incidence of malformations/skeletal variations, litter size, foetal body weights or sex ratios.
Stump 2001a	(OECD 2003; ANSES 2016; Risk Science Center 2018)	SD rats (25/group), Prenatal developmental tox study according to OECD 414, GLP	0, 3000, 6000, 10000 ppm (0, 226, 458, 747 mg/kg bw/d)	Diet, GD 0-20	NOAEL for maternal tox: 6000 ppm NOAEL for teratogenicity: 10000 ppm	Mean bw sign. decreased in HD between days 3-6 and 16-20 Mean liver weights sign. increased in HD Reproductive parameters unaffected No increased fetal mortality, bw, visceral malformations or skeletal malformations, but sign. increased incidence of pups with a 14th rudimentary rib in HD which is rather a variation than a malformation
Tox Services 2012	(ANSES 2016)	SD rats (5 animals) Supportive study	500 mg/kg bw/d	GD 12-19	NOAEL: 500 mg/kg bw/d	No effects were observed on pup weight, anogenital distance and genes associated with lipid, sterol, and cholesterol transport, steroidogenesis, intracellular lipid and cholesterol homeostasis, oxidative stress, insulin signalling, and transcriptional regulation.

Reference	Named in	Species, study type	Dosage	Administration, duration	NOAEL / LOAEL	Findings
Unnamed 2015	(ECHA 2023)	Rabbit (New Zealand White)	44, 135, 312 mg/kg bw/d	Diet, GD 6-29	135 mg/kg bw/d (maternal toxicity); 312 mg/kg bw/d (prenatal dev. toxicity)	Maternal toxicity at highest dose

Table V.31: Overview of studies regarding other effects

Reference	Named in	Findings
Stump 2002b	(OECD 2003; Risk Science Center 2018)	- 10 female rats per group were exposed via gavage to 0, 20, 200, 2000 mg/kg for 4 days to investigate estrogenic agonistic properties in an uterotrophic assay - No mortality, bw was decreased in HD, increased in positive control, uterine weights were sign. Increased only in positive control. - DEHTP was not an estrogenic agonist up to 2000 mg/kg
Eljezi et al. 2016	(Risk Science Center 2018)	- Cytotoxicity testing of phthalates in murine fibroblast L929 cells using cellular reduction of the colorimetric MTT reagent relative to positive and negative controls. - DEHTP demonstrated no significant differences compared with DMSO at any of the three concentrations tested (no further information given). - The only metabolite of DEHTP evaluated was MEHT. At a concentration of 0.05 mg/mL, MEHT was as toxic as MEHP, a primary metabolite of DEHP. - At 0.1 mg/mL, MEHT was as toxic as DEHP, and more toxic than MEHP. Across all the phthalates and metabolites tested, it was a general trend that metabolism of plasticizers increased their cytotoxicity.
(Poitou et al. 2022)		- in vitro study in a human microvascular endothelial cell line (HMEC-1 cells) to evaluate the cytotoxicity of DEHTP on cell function (viability, cell counting, total glutathione and intracellular adenosines) and mitochondrial function (mitochondrial respiration) - incubation with 40 µM (15 mg/L) continuous for 24-72 h or repeated (3h/d for three consecutive days) - glutathione content in the cells was determined to investigate oxidative stress - no signs of cytotoxicity, viability of the cells and glutathione content in the cells was not affected by continuous or repeated exposure
(Kambia et al. 2021)		- in vitro/in silico comparison of DEHTP metabolites on thyroid hormone receptors with those of DEHP - primary and secondary metabolites were synthesised - T-screen test was used as a rapid and functional test to assess interference with the T3 receptor with rat pituitary tumour cell line GH3 (ATCC, CCL-82.1) - oxidised metabolites of DEHTP had no effect on T3 receptors, while above 0.2 µg/ml 5-hydroxy-mono-(ethylhexyl)phthalate (5-OH-MEHTP) is primarily an agonist for thyroid hormone receptors with a synergistic effect - Monoesters (MEHP and mono-(2-ethylhexyl)terephthalate, MEHTP) were also active at the thyroid hormone receptor - According to authors, DEHTP is not able to interact directly with the thyroid hormone receptor

Part VI

Substance monograph for **diisononyl phthalate (DINP)** and derivation of HBM guidance values (HBM-GVs) for the general population

Technical reference

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Co-authors	Petra Apel, UBA, petra.apel@uba.de
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External Reviewers[3]	Austrian NH, Norwegian NH, Slovenian NH, UK NH, Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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[1] PU = Public

PP = Restricted to other programme participants (including the Commission Services)

RE = Restricted to a group specified by the consortium (including the Commission Services)

CO = Confidential, only for members of the consortium (including the Commission Services)

[2] WP/task co-leader(s) from the same WP

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Final version	May 2025	Review of AD 4.3 by EAA	Update of 24h bodyweight-related urine volume for adults and recalculation of HBM-GVs for adults. Removal of information on DNELs Clarification: ECHA, 2022 means ECHA registration dossier, 2022 Consideration of the current (January 2025) US EPA risk assessment on DINP Explicit justification why studies on endocrine effects of DINP were not considered as key studies Explanation that endocrine effects are covered by the selected TDI

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Abstract

Diisononyl phthalate (DINP) is a type of phthalate ester used primarily as a plasticizer. DINP is commonly used in the production of PVC (polyvinyl chloride) products such as cables, flooring, automotive parts, and toys. However, like other phthalates, DINP has raised concerns regarding its potential health effects, particularly its possible endocrine-disrupting properties.

Therefore, a systematic literature search was conducted at Pubmed and Scopus on DINP and its metabolites. Until May 2023, 626 publications were found, of which 342 were considered relevant. The most recent ones were further evaluated. In addition, existing authority reports were evaluated and the limit values derived from them were taken into consideration.

A toxicological (toxicity) reference value (TRV), the TDI of 0.15 mg/kg bw/d based on liver and kidney toxicity and derived by EFSA (2019) was used for the HBM-GV derivation, as this value also includes the effects of the critical study in the literature review as well as the assessment factors considered necessary by the authors of this report. Endocrine effects are covered by the numerical value of the selected TDI. The selected TDI is also supported by the US EPA's latest risk assessment from January 2025.

When calculating according to the urinary mass balance approach for individual major specific metabolites, the formula results for OH-MINP in a rounded HBM-GV of 0.45 mg/L urine for children and men and 0.39 mg/L urine for women[17]. For cx-MINP, the rounded HBM-GV is 0.44 mg/L urine for children and men and 0.38 mg/L urine for women. The rounded HBM-GV for the sum of both metabolites is 0.89 mg/L urine for children and men and 0.76 mg/L mg/L for women.

The derived HBM-GVs represent the concentration of the metabolite(s) in human urine up to which – for single substance evaluation – a risk for adverse health effects is not anticipated.

However, when assessing HBM results, it must be taken into account that exposure to mixtures of substances is usually present, so that the additional use of a MAF (mixture assessment factor/mixture allocation factor) or another suitable methodology should be considered for further assessments. At least structurally related substances or substances that display the same effect targets should be considered together for the next review and refinement of the HBM-GV.

Key Words

HBM guidance values, HBM-GV, diisononyl phthalate, DINP, OH-MINP, cx-MINP

[17] By using the outdated 24h bodyweight-related urine volume for men and women of 0.02 L/(kg bw x d) for the calculation of HBM-GV, the resulting $HBM\text{-}GV_{GenPop\ (adults)}$ would have been for the individual major specific metabolite OH-MINP 0.68 mg/L urine and for cx-MINP 0.66 mg/L urine. The HBM-GV for the sum of both metabolites would have been 1.34 mg/L for adults.

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We would like to thank the experts who provided us feedback on this work during the consultation period. Comments have been taken into account as far as possible. An overview of the substantive comments and how they were dealt with can be found in the annex to this deliverable comprising various reports.

Results

A TDI of 0.15 mg/kg bw/d derived by EFSA (2019) was used for the derivation of HBM-GVs for the major DINP specific metabolites monohydroxy isononyl phthalate (OH-MINP) and monocarboxy isononyl phthalate (cx-MINP). When calculating according to the urinary mass balance approach for individual major specific metabolites, the formula results for OH-MINP in a rounded HBM-GV of 0.45 mg/L urine for children and men and 0.39 mg/L urine for women. For cx-MINP, the rounded HBM-GV is 0.44 mg/L urine for children and men and 0.38 mg/L urine for women. The rounded HBM-GV for the sum of both metabolites is 0.89 mg/L urine for children and men and 0.76 mg/L for women.

Abbreviations and Acronyms

ALP	Alkaline Phosphatase
ALT	Alanine transaminase
AST	Aspartate aminotransferase
BBP	Benzyl butyl phthalate
BDI	Battelle Developmental Inventory
BfR	Bundesinstitut für Risikobewertung
BMI	Body mass index
C	Carbon
CAS	Chemical Abstracts Service
CHO	Chinese hamster ovary
DBP	Dibutyl phthalate
DEHP	Di(2-ethylhexyl) phthalate
DiBP	Diisobutyl phthalate
DIDP	Diisodecyl phthalate
DINP	Diisononyl phthalate
DnBP	Di-n-butylphthalate
DNEL	Derived no effect level
DPHP	Di (2-propylheptyl) phthalate
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
F_{ue}	Urinary excretion factor
GLP	Good Laboratory Practice
HBM	Human biomonitoring
HBM-GV	Human biomonitoring guidance value
IUPAC	International Union of Pure and Applied Chemistry
LOAEL	Lowest observed adverse effect level
MCEP	Monocarboxyethyl phthalate
MCIBP	Monocarboxy isobutyl phthalate
MCIHXP	Monocarboxy isohexyl phthalate
MCIOP	Monocarboxy isooctyl phthalate
MHINP	Monohydroxy isononyl phthalate
MINP	Monoisononyl phthalate
MNCL	Mononuclear cell leukaemia
MOE	Margin of exposure

MOA	Mode of action
MOINP	Monooxoisononyl phthalate
MW	Molecular weight
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NSRL	No significant risk level
OECD	Organisation for Economic Co-operation and Development
OECD SIDS	OECD Screening Information DataSet
PCNA	Proliferating-cell nuclear antigen
PND	Postnatal day
POD	Point of departure
PPAR	Peroxisome proliferator-activated receptor
PU	Polyurethane
PVC	Polyvinyl chloride
PXR	Pregnane X receptor
QSAR	Quantitative Structure Activity Relationship
SHE	Syrian hamster embryo
TDI	Tolerable daily intake
TG	Test guideline
TRV	Toxicological (toxicity) reference value
US EPA	U.S. Environmental Protection Agency

1 Introduction

Phthalates are esters of phthalic acid (1,2-benzenedicarboxylic acid) and are mainly used as plasticisers to increase the flexibility, transparency, durability and longevity of plastics. A well-known representative is diisononyl phthalate (DINP). DINP is primarily a general-purpose plasticiser. More than 90% of the DINP produced is used to improve the flexibility, pliability and resilience of a wide range of polyvinyl chloride (PVC) products, including vinyl flooring, insulating mesh for wires and cables, gloves, toys, pipes, garden hoses, artificial leather, shoes artificial leather, footwear, underbody protection for cars and roofing materials. Non-PVC products containing DINP are rubber, inks, pigments, paints, varnishes, adhesives and sealants. Exposure to the public may be possible through the use of PVC or non-PVC products containing DINP and to workers in the workplace (Tomar et al., 2013).

DINP is not a single compound, but a complex mixture of isomers. Therefore, there are assigned two different CAS registry numbers for DINP, 28553-12-0 and 68515-48-0, which indicate different starting alcohols. In CAS 28553-12-0, more than 50% of the alcohol component consists of alkyl-substituted octanols or heptanols or hexanols. DINP with CAS registry number 68515-48-0 is produced by the so-called polygas technology and contains approximately equal amounts of 3,4-; 4,6-; 3,6-; 3,5-; 4,5- and 5,6-dimethylheptanol-1. However, both isomers share common constituents and cannot be differentiated through their physicochemical properties. The toxicological data and effect profiles also show essentially no differences, so that both isomers are to be treated equally in toxicological and regulatory terms (BAuA, 2001; Environment Canada and Health Canada, 2015).

In the context of the REACH registration, the total tonnage for DINP is given as 10^5 - 10^6 tons per year (https://chem.echa.europa.eu/100.044.602/dossier-list/reach/dossiers/active?searchText=DINP).

2 Methodology

Relevant agency reports and risk assessments for DINP were identified by general desk research.

For a systematic literature search of scientific publications, a search string was developed consisting of (A) a health part and (B) synonyms for DINP and its major metabolites (Table IX.69). The full search term is shown in the Appendix.

Table VI.32: Parts of the search term used to search for scientific publications

Health part	health OR adverse OR toxic* OR neurotox* OR expos* OR epidem* OR cohort OR genotox* OR genetic* OR carc* OR cancer OR reproduc* OR reprotox* OR developmental OR fertil* OR disease OR neurologic* OR metabol* OR ADME OR PBPK OR PBTK OR excret* OR “in vivo” OR "in vitro" OR "in silico" OR endocrine OR "half-life" OR immun* OR MOA OR "mode of action"
Synonyms for DINP and its major metabolites	DINP OR "Diisononyl phthalate" OR “Di-isononyl phthalate” OR MINP OR “Mono-isononyl phthalate OR 7OH-MMeOP” OR “Mono(4-methyl-7-hydroxyoctyl) phthalate” OR 7oxo-MMeOP OR Mono(4-methyl-7-oxooctyl) phthalate OR 7cx-MMeOP OR “Mono(4-methyl-7-carboxyheptyl) phthalate“ [OR *additional synonyms of all substances*]

The search was carried out in SCOPUS and Pubmed and revealed a total number of 626 publications that were published until May 2023. The publications were screened for relevance according to title and abstract using DistillerSR. In this first step, 284 publications were excluded. Of the remaining 342 publications the most recent ones (January 2013 – May 2023) were screened by full text and relevant information are discussed in the following chapters of this report.

The following exclusion criteria were applied:

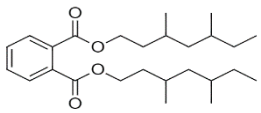
- No information on DINP or its metabolites
- Study type not of interest (e.g. analytical methods, removal, laboratory experiments)
- Studies with no information relevant for the derivation of human biomonitoring values

3 General characteristics

3.1 Physico-chemical properties

The information on physico-chemical properties of DINP is based on the ECHA registration dossier (2022).

Table VI.33: Substance characteristics and physicochemical properties of DINP

Names	Diisononyl phthalate Di-iso-nonyl phthalate Phthalic acid diisononyl ester DINP
IUPAC name	1,2-bis(7-methyloctyl) benzene-1,2-dicarboxylate
EC no.	249-079-5
CAS no.	28553-12-0, 68515-48-0
Sum formula	C_{26} H_{42} O_4
Structural formula	
Appearance [1013 hPa; at 20°C]	colourless liquid
Molecular weight [g/mol]	418.62
Relative density [g/cm³; at 20°C]	0.97
Melting point [°C; 1013.25 hPa]	< - 50
Boiling point [°C; 1013 hPa]	> 300
Vapour pressure, Pa [at 20°C]	0.00006
Log P_{OW} [25°C, pH 4.6]	8.8-9.7
Water solubility [µg/L; at 20°C]	0.6

3.2 Use of the substance

The use of DINP is increasing, not least because it can be used as a substitute for DEHP. In 2021, market reports have calculated an overall global market share of 80.6% for phthalate plasticisers, of which DINP had the largest market share in terms of volume at 28.6% (MarketsAndMarkets, 2023). DINP is used in the following products: Adhesives and sealants, coating products, lubricants and greases, and polymers.

Releases of DINP to the environment are likely from indoor uses (e.g. machine detergents/cleaners, car care products, paints and coatings or adhesives, fragrances and air fresheners), outdoor uses, indoor uses in closed systems with minimal release (e.g. cooling fluids in refrigerators, oil-based electric heaters) and outdoor use in closed systems with minimal release (e.g. hydraulic fluids in vehicle suspensions, lubricants in engine oil and brake fluid (ECHA registration dossier, 2022).

A major use of DINP is in the production of plastic materials such as PVC, polyurethane (PU) or polyester (PS). Further uses of DINP are traffic cones, sex toys, erasers, packaging for cosmetic products, carpet blanking, roofing membranes, hoses, vinyl flooring, Christmas decorations, inks for printing, electronic products, batteries, artificial leather, footwear, jewellery, clothing articles, toys, erasers, balls, baby products and baby equipment, baby furniture, sporting articles, rubber and plastic items, headsets, air mattresses, medical devices and many more (Danish EPA, 2013; Environment Canada and Health Canada, 2015). According to ANNEX XVII to REACH, entry 52, the use of DINP is restricted in the EU. It shall not be used as substances or in mixtures, in concentrations greater than 0,1 % by weight of the plasticised material, in toys and childcare articles which can be placed in the mouth by children. Such toys and childcare articles containing these phthalates in a concentration greater than 0,1 % by weight of the plasticised material shall not be placed on the market (https://echa.europa.eu/documents/10162/0923c10f-5495-16ee-5c0f-dd3613fbde6d).

As of December 2004, the use of DEHP and DINP in all cosmetic products in the EU was restricted by Directive 2004/93/EC adapting Annexes II and III of Directive 76/768/EEC (UBA, 2012).

3.3 Possible routes of exposure

No natural sources of DINP are known (Environment Canada and Health Canada, 2015). As mentioned above, DINP is used in and can be released from various consumer products. The release of DINP has been reported to occur during manufacture and processing of the substance, including transportation and storage of materials, and in the manufacture, use and disposal of products containing DINP (Environment Canada and Health Canada, 2015).

On behalf of the German Environment Agency (UBA), the German Federal Institute for Risk Assessment (BfR) has estimated several sources of exposure to phthalates using the example of bis(2-ethylhexyl) phthalate (DEHP) and DINP (UBA, 2012). DINP was found mainly in juices. In another study, DINP was the most prevalent plasticiser among various plasticisers analyzed in olive oil and was originating mainly from the production process itself, except for prolonged storage in plastic bottles (Freitas et al., 2024).

In addition to food, house dust plays an important role in human exposure to DINP. DINP has been detected in settled house dust by various studies (Kubwabo et al., 2013; Fromme et al., 2013; Plichta et al., 2019). The presence of DINP in house dust may be primarily attributed to mechanical abrasion, with only a minimal fraction existing in the gaseous phase due to its low vapor pressure. Phthalates in house dust can be absorbed by inhaling fine particles (relatively low contribution to aggregated exposure) or orally (especially relevant for children). Children, especially infants, can be more exposed to phthalates than adolescents and adults. They are exposed to plasticisers not only through food, but also increasingly through house dust and things they put in their mouths (called “mouthing”).

Based on a screening analysis of professional floor installers, the upper limit of DINP exposure from the installation of vinyl flooring containing 9% DINP is 151 µg/day. This exceeds the NSRL (No Significant Risk Level) for DINP, which is 146 µg/day (Office of Environmental Health Hazard Assessment, 2016).

According to a cumulative risk assessment of five phthalates in European children and adolescents, there is a trend for several phthalates in recent years to decrease considerably over time in European adolescent and children population with no significant influence of sex or age but this does not account for DINP. However, according to the authors, the contribution of DINP to the hazard indices of phthalate mixtures was negligible when compared to the phthalates DnBP, DiBP and DEHP (Lange et al., 2022).

4 Toxicological (toxicity) reference values proposed by European or relevant non-European institutions or registrants

The following table summarises available toxicological reference values.

Table VI.34: Available toxicological (toxicity) reference values.

Agency	Type of value	Value	Key study	Exposure route	Critical effect	POD	AFs	Source
EFSA	TDI	150 µg/kg bw/d	Exxon Biomedical Sciences, 1986	Oral (diet)	Chronic hepatic & renal effects	NOAEL = 15 mg/kg bw/d	100 (uncertainty factor for NOAEL)	EFSA, 2019
California EPA	NSRL	146 µg/d	unknown[18]					California Office of Environmental Health Hazard Assessment, 2016
Dutch Consensus Group, RIVM,1998	Refer to entry "EFSA" as the Dutch Consensus group refers to the same key studies and endpoints.							
Health Canada	ADI	290 µg/kg bw/d		Oral				Health Canada 1998
CHAP (CPSC)	ADI	120 µg/kg bw/d	Lington et al., 1997	Oral	Spongiosis hepatis	NOAEL = 15 mg/kg bw/d	100-fold combined uncertainty/adjustment	CPSC 1998

[18] A NSRL of 146 µg/d was derived as stated in the report (California Office of Environmental Health Hazard Assessment, 2016). However (California Office of Environmental Health Hazard Assessment, 2016) only referenced (California EPA et al., 2013) which summarized the studies used in the derivation of the value. As such no detailed information on the derivation are present. It is only known from (California Office of Environmental Health Hazard Assessment, 2016) that the NSRL is defined as the level of exposure that would result in no more than one excess case of cancer in 100,000 individuals exposed to the chemical over a 70-year lifetime.

Agency	Type of value	Value	Key study	Exposure route	Critical effect	POD	AFs	Source
US EPA	MOE		Lington et al., 1997	Oral	Liver toxicity	NOAEL = 15 mg/kg bw/d	Human equivalent dose (HED): 3.5 mg/kg bw/d "A total UF of 30 was selected for use as the benchmark MOE (based on an interspecies UF (UFA) of 3 and an intraspecies UF (UFH) of 10)."	US EPA, 2025 (https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0436-0137)

5 Toxicological profile

DINP is not harmonised classified according to CLP in the EU. The majority of self-classification by industry propose no classification, while one notifier indicate Repr. 2. (H361) and Resp. Sens. 1A (H334). However, the RAC opinion did not confirm classification for reprotoxicity (RAC, 2018)[19].

5.1 Findings in humans

5.1.1 Toxicokinetics and metabolism

DINP is metabolized by hydrolysis of phthalic acid and side chain oxidation of the ester group. A metabolic transformation pathway for DINP was proposed by Saravanabhavan and Murray (2012) (California EPA et al., 2013). MINP is the monoester metabolite of DINP.

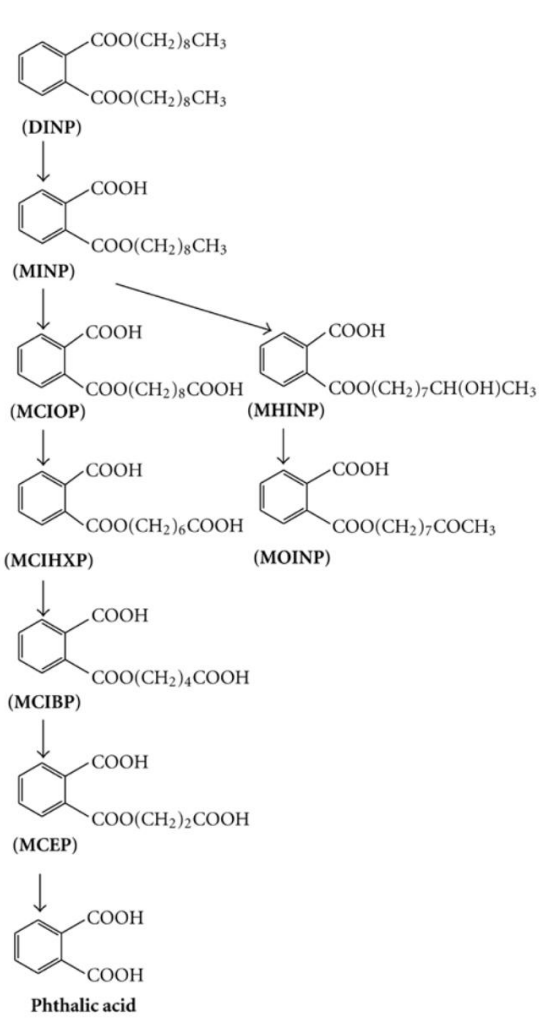


Figure VI.3: Proposed metabolic transformation pathway for DINP. DINP (M=418.62 g/mol): diisononyl phthalate; MINP (M=292.4 g/mol): monoisononyl phthalate; MCIOP (carboxy-MINP) (M=336.4 g/mol): monocarboxy isooctyl phthalate; MHINP (OH-MINP) (M=308.374 g/mol): monohydroxy isononyl phthalate; MCIHXP (M=294.1 g/mol): monocarboxy isohexyl phthalate; MOINP (oxo-MINP) (M=306.358 g/mol): monooxoisononyl phthalate; MCIBP (M=266.08 g/mol): monocarboxy isobutyl phthalate; MCEP (M=238.19 g/mol): monocarboxyethyl phthalate (California EPA et al., 2013).

[19] https://echa.europa.eu/documents/10162/56980740-fcb6-6755-d7bb-bfe797c36ee7

There are two relevant toxicokinetic studies in humans available. In the first human toxicokinetic study, 43.6% of the orally administered dosage was recovered in urine as DINP metabolites within 48 hours: 20.18% as OH-MINP, 10.68% as cx-MINP, 10.60% as oxo-MINP, and 2.15% as MINP (Koch and Angerer, 2007). The elimination pattern was multi-phase; elimination half-lives in the second phase (starting 24 h post-dose) can only be calculated to be 12 h for OH- and oxo-MINP metabolites and 18 h for cx-MINP metabolites.

However, the toxicokinetics in this study were only investigated in a single male volunteer (senior author of the study), who received a single oral dose of 1.27 mg/kg bw of deuterium-labelled DINP. As toxicokinetics are subject to considerable individual variability, it is difficult to draw general conclusions from this individual measurement. The variability can depend on the age or sex of the test person but can also vary in supposedly similar population groups. Furthermore, healthy test subjects are used for such studies indicating that the data is not representative of the entire population.

In contrast, Anderson et al. (2011) conducted a study, according to an ethically approved protocol, in ten male and female subjects being exposed to a single oral dose of 0.012 mg/kg bw and 0.121 mg/kg bw/d of deuterated DINP (D4-DINP) in two study periods. More than 90% of the metabolites were excreted in the first 24 hours of urine collection, the remainder in the second 24 hours. Total metabolites were 32.9±6.4% of the administered dose. Fractional excretion of primary and secondary specific metabolites was 3.1%, 6.6%, 12.3% and 10.9% for MINP, 7-MOINP, 7-MHINP and 7-MCIOP, respectively. The estimated elimination half-lives of the metabolites were between four and eight hours (Anderson et al., 2011 as cited in (California EPA et al., 2013; United States Environmental Protection Agency, 2022)).

Due to the significantly higher group size with two sexes, the data from Anderson et al. (2011) are considered to be significantly more robust than those from Koch and Angerer (2007). The data from Anderson et al. are therefore used for the further derivation of the threshold values.

Study summaries and further information are given in Table VI.4 (Appendix – Toxicological profile

No relevant PBPK models have been identified in the literature.

5.1.2 Effects on skin and mucous membrane

There are two studies available with human volunteers who participated in a skin irritation and a skin sensitization test (Hill Top Research 1995) as cited in (NICNAS, 2012). In the skin corrosion test, the substance was applied undiluted on the skin for 24 hours, followed by an observation period of 24 hours. No adverse effects were observed. In the positive control group mild to moderate erythema was observed but not with the test substance.

The skin sensitisation study with 28 subjects in the pilot study and 76 subjects in the definitive study was negative as well. After induction applications being made three times a week for three successive weeks and a challenge after 10-17 days, no signs of skin sensitisation were observed.

5.1.3 Epidemiological studies on DINP published between 2018 and 2023

For its current risk assessment on DINP (https://www.federalregister.gov/d/2025-00730), EPA has compiled, among other things, references to epidemiological studies on DINP published between the beginning of 2018 and the end of 2023. After filtering out references that only assessed exposure using a biomarker matrix other than urine or breast milk without any other exposure assessment, 49 references remained (https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0436-0065) and proceeded to data quality evaluation. As a result, none of the studies could be used for a quantitative dose-response assessment, among other things because the study design was not suitable or because mixtures of substances were present and no solitary exposure to DINP. Rather, the studies were regarded to provide qualitative support within the weight of scientific evidence. Detailed assessments can be found here: https://www.epa.gov/system/files/documents/2025-01/09-.-dinp-.-data-eval-for-human-health-haz-epi-.-public-release-.-jan-2025.pdf.

5.2 Animal findings and *in vitro* studies

5.2.1 Toxicokinetics and metabolism

Several toxicokinetic studies are available in rats (6 studies) and mice (1 study) with exposure via the oral, intravenous or dermal route of exposure. Studies are summaries in Table IX.75 (Appendix). In a study, total radioactivity in faeces, urine, blood and various tissues of rats was measured after oral administration of [^{14}C]DINP to male and female rats (50 or 500 mg/kg bw/d for one day or 50, 150 or 500 mg/kg bw/d for five consecutive days). Radioactivity was mainly recovered in the gastrointestinal tract, liver and kidneys. After a single dose, absorption was found to decrease with increasing dose with 49% of the low dose of 50 mg/kg bw/d vs. 39% of the high dose of 500 mg/kg bw/d being excreted in the urine within 72 hours.

On the other hand, the proportion in the faeces increased slightly from 50% in the low dose group to 52% in the high dose group (McKee et al. 2002) as cited in (Environment Canada and Health Canada, 2015).

Elimination half-life in rats was 364 ± 146 min for i.v. and 150 ± 58 min after oral exposure with a low oral bioavailability of about 1.19 %. The total excretion of DINP was 13.64 ± 3.99 % in the faeces after 48 hours (Hou et al. 2013).

When administered to pregnant rats, the most predominant metabolite found in urine was MCIOP, while amounts of MINP and DINP were almost negligible. However, in maternal plasma, all metabolites were present (Clewell et al., 2013 and Silva et al., 2006) as cited in (Environment Canada and Health Canada, 2015).

According to (Environment Canada and Health Canada, 2015), metabolites that can appear in animals after oral exposure are Monoisononyl phthalate (MINP), its glucuronidated form (MINP-Gluc), Mono(hydroxyisononyl)phthalate (MHINP), Mono(oxoisononyl) phthalate (MOINP), Mono-carboxy-isooctyl phthalate (MCIOP), Mono(carboxy-isoheptyl) phthalate (MCIHpP), Mono-(3-carboxypropyl) phthalate (MCPP), Mono-n-octyl phthalate (MnOP), Phthalic Acid (PA).

5.2.2 Acute toxicity with single administration

The acute toxicity of DINP is low. There are six acute toxicity studies available for the oral route in rats, two for inhalation exposure in rats and one study for dermal exposure in rabbits. Studies are summarised in Table IX.76 (Appendix). No deaths occurred in any of the studies. For the oral route of exposure up to 50,000 mg/kg bw were tested (NICNAS, 2012; United States Environmental Protection Agency, 2022), dermal toxicity was investigated up to 3,160 mg/kg bw (NICNAS, 2012) and inhalation toxicity up to 4.4 mg/L (NICNAS, 2012; United States Environmental Protection Agency, 2022).

5.2.3 Subacute, subchronic and chronic toxicity

DINP has been investigated in a large number of studies with repeated exposure in mice, rats, rabbits, dogs and monkeys with oral exposure via the diet or by gavage. Studies are summarised in Table IX.77 (Appendix). In almost all studies, liver and kidney effects were detected, which were reflected in increased organ weights and altered clinical chemistry parameters. In long-term studies, the observations were accompanied by histopathological changes.

There are two 2-year dietary key studies with DINP reported in (ECHA, 2013). Both the Aristech study (1994) and the Exxon study (1986) found a statistically significant increase in the incidence of spongiosis hepatis, serum liver transaminases, and liver and kidney weights at the LOAEL determined in the studies (359-442 and 152-184 mg/kg bw/d in males and females for the Aristech and Exxon study, respectively). The incidence of spongiosis hepatis in females was similar and low for all control and substance doses used in both studies. In males, it was clearly treatment related and dose dependent in both studies.

In the first key study by Exxon (1986) a NOAEL of 0.03 %, corresponding to 15-18 mg/kg bw/d, was established. It was based on a slightly reduced survival rate in females, an increased incidence of spongiosis hepatis and increased serum levels of liver transaminases in males (1.5-2x), an increase in absolute and relative liver (11-19%) and

kidney weights (5-10%) in both sexes and a 61% increase in relative and absolute spleen weights at 0.3 % in males. The rats exhibited enlarged livers and a small increase in liver enzymes (ALT, AST, ALP).

In the second key study in rats by Aristech (1994) a NOAEL of 0.15 %, corresponding to 88 - 109 mg/kg bw/d could be established. It was based on increased ALT and AST values in the liver, absolute and relative increases in liver and kidney weights in both sexes and other histopathological findings indicating liver toxicity. At the two highest doses, i.e. approx. 359 and 733 mg/kg bw/d, a marked increase in spongiosis hepatis was also observed in the male animals (ECHA, 2013).

According to ECHA, there were differences in the methodology of both studies as more liver sections were examined in the Exxon study than in the Aristech study. Therefore, the Exxon study with a NOAEL of 15 mg/kg bw/d was considered more reliable (ECHA, 2013). The effects responsible for deriving the NOAEL were also considered by EFSA to be the most critical effect and were subsequently used to derive a tolerable daily intake (TDI) (EFSA, 2019).

Similar results in the livers and kidneys were observed in a 2-year mice study by Moore (1998b) with dietary concentrations of 0, 500, 1500, 4000 or 8000 ppm, corresponding to 0, 90/112, 276/336, 742/910 or 1560/1888 mg/kg bw/d for males and females, respectively. Effects included decreased absolute and relative kidney weights throughout the study, increased incidence of liver masses in males, and increased absolute liver weights and significantly decreased mean body weight gain in females. The US EPA concluded a NOAEL of 90 mg/kg bw/d for this study (United States Environmental Protection Agency, 2022).

5.2.4 Effects on skin and mucous membranes

There are three studies available on skin irritation and two on eye irritation. Studies are summarised in Table 79 (Appendix). Undiluted DINP was applied to abraded skin of rabbits and left for 24 hours. Only slight erythema and oedema were observed and the effects were reversible (BASF 1981b, Hüls 1985b, ECB 2003) as cited in (NICNAS, 2012). Similar results were observed with undiluted DINP applied for 4 hours to the clipped intact skin of six male New Zealand white rabbits with a semi-occlusive dressing, followed by an observation period of 72 hours followed by very slight reversible erythema in one rabbit at 1 h and another at 24 h (Exxon Biomedical Sciences 1996a, ECB 2003) cited in (NICNAS, 2012). Minimal skin and eye irritating properties have also been identified by CPSC (2010) as cited in (United States Environmental Protection Agency, 2022). Six rabbits being exposed to a single 0.1 ml undiluted ocular installation of DINP into the left conjunctival sac, developed irritation responses with limited to slight conjunctival redness during the first 24 hours. All responses had resolved within 48 hours (Unnamed 1968) cited in (ECHA registration dossier, 2022).

DINP is therefore described as minimally irritating to eyes and skin by the ECHA and US EPA (ECHA, 2013; United States Environmental Protection Agency, 2022).

Regarding skin sensitisation, there are two Buehler tests available. In one test with 40 animals (20 treated + 20 ctrl), no signs of sensitisation were observed, in another study some evidence of sensitisation and a score 2 erythema was observed in 3/20 animals with score 1 in 4 animals from the control group. In further studies which were not performed according to standards, there were signs of inflammation with adjuvant effect on IgE and IgG. However, the United States Environmental Protection Agency concluded in 2020, that DINP is not a dermal sensitizer (United States Environmental Protection Agency, 2022). The ECHA concluded that DINP lacks intrinsic sensitising potential but could be a possible contributor to the increasing prevalence of atopic (IgE-mediated) allergic diseases (ECHA, 2013).

5.2.5 Genetic toxicity

The genotoxicity of DINP has been investigated in a total of 20 tests from several test systems. The test systems are the Salmonella reverse mutation test, the mouse lymphoma cell mutation test, the chromosome aberration test on Chinese hamster ovary (CHO) cells and the *in vivo* micronucleus test in rats and mice. DINP gave clear negative results in all tests with and without metabolic activation. Studies are summarised in Table VI.41

According to the (United States Environmental Protection Agency, 2022) the genotoxicity of DINP metabolites has not been directly investigated. Nevertheless, the genotoxicity of DINP was investigated in the presence of metabolic activating systems.

Positive results were obtained in a single *in vitro* transformation study of Balb/c 3T3 cells.

Furthermore, a cell transformation assay was performed. These are used to detect a change in the growth pattern of cells that indicates loss of contact inhibition, which is characteristic of cancer cells. A summary of the studies reviewed by EC JRC (2003) is described in (California EPA et al., 2013). In the early 1980s, seven *in vitro* cell transformation studies were performed on Balb/c 3T3 A31 mouse cells. All of the studies examined were performed without metabolic activation.

The three studies conducted by Litton Bionetics (1981b, 1981c and 1985b) were negative. One of the four studies performed by Microbiological Associates was positive (Microbiological Associates 1981c) for the induction of type II transformed foci with a more orderly and defined edge than type III foci and type III transformed foci that appear as dense, multi-layered foci with random orientation at the foci edge and invasion into the surrounding monolayer, compared to acetone negative controls. In this study, three, six and twelve type III foci were examined at doses of 0.03, 0.3 and 1.0 µl/ml, respectively. These increases were statistically significant and presumably dose-dependent. A more recent study by Barber et al. (2000), also performed on Balb/c 3T3 A31 mouse cells without metabolic activation, was negative for cell transformation. DINP has not been tested for induction of cell transformation in other cell types, such as Syrian hamster embryo (SHE) or C3H/10T1/2 cells.

However, the weight of evidence of all tests supports the conclusion that DINP is not genotoxic (United States Environmental Protection Agency, 2022).

5.2.6 Carcinogenicity

Chronic toxicity data for DINP are available from several exposure studies conducted in rats and mice via the oral route. No chronic exposure data on DINP are available for humans or primates. Studies are summarised in

Table IX.81

In total, in various studies, statistically significant increases in many tumour types were observed in rats and mice, such as an increase in hepatocellular tumours, hepatocellular adenomas and carcinomas, splenic mononuclear cell leukaemia and renal tubular cell carcinomas. Some animals also showed a non-significant increase in tumour types that are considered rare and/or uncommon, such as renal tubular cell carcinoma, pancreatic islet cell carcinoma, testicular interstitial (Leydig) cell carcinoma and uterine adenocarcinoma.

The following tumours occurred: Liver tumours in female SD rats, as well as male and female F344 rats and B6C3F1 mice, mononuclear cell leukaemia (MNCL) in F344 rats of both sexes, renal tubular cell carcinomas in male F344 rats, which is a rare tumour disease, and also rare pancreatic islet cell carcinomas in male SD rats and female B6C3F1 mice. In SD rats, testicular Leydig cell carcinomas were also rarely observed in the male and uterine carcinoma in females.

In a re-evaluation of both studies where increased incidences of MNCL were observed it was concluded that MNCL commonly occur in F-344 rats and that there is evidence for the existence of a threshold at a level that is not biologically relevant and which must be exceeded before carcinogenic effects can be observed. It was therefore concluded that the data is not sufficient to assume a carcinogenic hazard to humans (Caldwell et al., 1999). However, according to ECHA the increased incidence of MNCL in some studies is difficult to interpret given the high and variable background incidences and the unclear relevance to humans (ECHA, 2013).

In addition, (Caldwell et al., 1999) conducted a histopathological re-evaluation of formalin-fixed kidneys after 6- and 12-month sacrifices from the study by (Lington et al., 1997), in which renal tumours were found. The aim was to determine the alpha 2µ-globulin status in these samples and to investigate cell proliferation by immunohistochemical detection of the proliferating-cell nuclear antigen (PCNA) marker. The authors observed that treatment with DINP had resulted in a dose-dependent accumulation of alpha 2µ-globulin in male rat kidneys concomitant with an increase in cell proliferation in these areas and concluded that the original renal tumour findings are likely explained by an alpha 2µ-globulin-dependent mechanism and were not regarded relevant for humans.

It should be kept in mind as well that DINP, similar to most of the phthalates, has shown to be negative in a number of genotoxicity assays and that until now it is not classified as being carcinogenic by any international authority. For example, the kidney tumours were assessed by ECHA as not relevant to humans, as the neoplasms that occurred were assessed as alpha-2µ-globulin mode of action. For liver tumours, peroxisome proliferation was suspected, which was not fully elucidated (ECHA, 2013).

However, a non-genotoxic mechanism would be possible. According to the US EPA (2021) DINP binds to PPAR and activates the rodent as well as the human PPARα and PPARγ but not PPARβ receptors. In addition, DINP has been shown to activate the human constitutive androstane receptor (hCAR2) and pregnane X receptor (PXR), and the metabolites, particularly MINP, activate the hCAR2 isoform, suggesting that DINP and its metabolites have more than one mechanism of action. DINP has also been shown to promote and induce tumorigenesis in a variety of cell types through aryl hydrocarbon receptor -mediated genomic and non-genomic pathways. DINP induces several changes in rodent liver consistent with PPARα activation (United States Environmental Protection Agency, 2022).

US EPA published recently (January 2025) the following conclusion: “DINP has been evaluated for carcinogenicity in two 2-year dietary studies of F344 rats (Covance Labs, 1998b; Lington et al., 1997), one 2-year dietary study of SD rats (Bio/dynamics, 1986), and one 2-year dietary study of B6C3F1 mice (Covance Labs, 1998a). Across available studies, treatment-related hepatocellular adenomas and carcinomas have consistently been observed in F344 and SD rats as well as B6C3F1 mice. Existing assessments of DINP by U.S. CPSC (2014, 2010), Health Canada (ECCC/HC, 2020; EC/HC, 2015; Health Canada, 2015), ECHA (2013), and NICNAS (2012) have postulated that DINP causes liver tumors in rats and mice through a PPARα MOA. Consistent with EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005) and the IPCS Mode of Action Framework (IPCS, 2007), EPA further evaluated the postulated PPARα MOA for liver tumors, as well as evidence for other plausible MOAs for DINP. Although some uncertainties remain, there is strong evidence to support the postulated, non-genotoxic, PPARα MOA. Under the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), EPA determined that DINP is Not Likely to be Carcinogenic to Humans at doses below levels that do not result in PPARα activation (KE1). Further, the non-cancer

chronic POD (NOAEL/LOAEL of 15/152 mg/kg bw/day) based on non-cancer liver effects; see EPA's Non-cancer Human Health Hazard Assessment for Diisononyl Phthalate (DINP) (U.S. EPA, 2025) will adequately account for all chronic toxicity - including carcinogenicity - which could potentially result from exposure to DINP. Therefore, the noncancer chronic POD of 15 mg/kg bw/day is considered protective of PPARα activation and carcinogenicity." (https://www.epa.gov/system/files/documents/2025-01/21.-dinp-.-hhh-cancer-tsd-.-public-release-.-hero-.-jan-2025.pdf)

5.2.7 Reproductive toxicity

Fertility

Data on fertility effects of DINP is sparse. An overview on existing studies dealing with effects on fertility in animals can be found in Table IX.82

There are two key studies that were conducted in accordance with the US EPA guidelines. These are a one- and a two-generation study in rats by Waterman et al. (2000) as cited in (BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022). The studies were GLP-compliant.

The one-generation study was conducted as a pilot study for the two-generation study in order to determine suitable dosages. In both studies 30 male and female SD rats were exposed per dose group through the diet 10 weeks before mating until lactation. In the one-generation study the rats were exposed to 0, 0.5, 1.0, 1.5 %, corresponding to 0, 301–923, 622–1731, 966–2246 mg/kg bw/d. In the two-generation study concentrations of 0.0, 0.2, 0.4, or 0.8 %, corresponding to 0, 114–395, 235–758, 467–1541 mg/kg bw/d were used.

Absolute testes weights were significantly increased in the one-generation study, ovarian weights were significantly decreased at 1.5 %. No adverse effects on mating or fertility were identified. In the two-generation study no increased mortality of the parental generation could be observed but at 0.8% a significantly decreased body weight was observed at PND 14 and 21. The sex ratio and survival rate during lactation were not affected in the F1 generation. The average litter sizes of the treated groups were significantly larger (18 to 21 %) than those of the control group. The weight of the male offspring from the 0.8 percent group was significantly reduced at PND 0. Significant reductions in body weight gain of the offspring were also observed at 0.4 and 0.8 % on days 7, 14 and 21.

No increased mortality or treatment-related symptoms were observed in treated animals at P2. At the time of selection (approx. 6 weeks after weaning), dose-dependent decreases in body weight gain were observed in 0.4 % and 0.8 % of males and females, respectively. Females in the 0.8 % group consistently showed lower body weight gain than the control group during the pre-oestrus (significant for the first 2 weeks), gestation (non-significant) and lactation (significant for PND 4 to 21). There were no significant effects on the standard reproductive indices. Offspring weight gain after PND 0 in the F2 generation was not significantly different from controls when differences in litter size were taken into account. Offspring weights were significantly reduced at 0.4 % and 0.8 % during lactation. No effects on reproductive organ weights or histopathology were observed. At necropsy, no gross effects on the P2 animals or their offspring were observed.

In addition, there are some more recent studies, although these are not guideline-compliant and in some cases the focus of the investigation was on very specific parameters like impaired glucose tolerance or plasma adipokine levels as described in (J. Chen et al., 2022; Earl Gray, 2023; Neier et al., 2019, 2020).

Some studies also found effects on normal fertility parameters, although these were not dose-dependent and showed considerable variability and therefore could not be clearly attributed to the treatment (C. Chiang et al., 2020; C. Chiang & Flaws, 2019; L. R. Chiang et al., 2020). In addition, some of the studies referred to mixtures of several phthalates, so that here too it is not possible to assign the effects clearly (Amjad et al., 2021; Barakat et al., 2019).

Statistically significant effects on fertility parameters were determined by (Boberg et al., 2016, 2011), but the statistical significance of the effects was questioned by (M. Chen et al., 2017).

In both key studies, no significant effects on fertility could be observed. This evaluation is in line with those of authority reports by (ECHA, 2013; United States Environmental Protection Agency, 2022).

Anti-androgenic effects on reproductive function were observed by (Boberg et al., 2016, 2011) but were considered smaller than those of DEHP, DBP or BBP. According to (United States Environmental Protection Agency, 2022) there is evidence for antiandrogenic effects of DINP like decreased AGD and decreased foetal testicular testosterone content, presumably due to decreased production, which are consistent with an anti-androgenic mode of action. According to the ECHA, DINP has anti-androgenic potency due to *in vivo* decreased foetal testicular testosterone concentration but may also exhibit its effects through other modes of action (ECHA, 2013).

In a review article, the effects of, among others, DINP on the male germ cells and the offspring were investigated. According to the authors, *in vitro* studies showed weak oestrogenic effects, while in vivo anti-androgenic effects were prominent. In addition, a reduced sperm count, an increased frequency of abnormal sperm and DNA damage in germ cells, especially after chronic exposure and when immature animals were exposed, were found. According to the authors, the phthalates studied could cause mutations in male germ cells leading to increased prenatal and postnatal mortality of the offspring and the occurrence of congenital malformations, growth retardation, delays in sexual development, shortening of the anogenital distance in males, disturbances of the sex ratio and reduced semen quality in the F1 generation (Dobrzyńska, 2016).

Developmental toxicology

The developmental toxic effects of DINP have been investigated in a large number of studies. Studies are summarised in

Table IX.83 In a recent evaluation of the studies by the US EPA it was concluded that the available data shows a consistent pattern of effects.

The information that is currently available about developmental toxicity typically demonstrates a regular pattern of effects across distinct exposure windows (in utero, prenatal, and post-natal exposure). The findings of the one- and two-generation reproductive studies show that DINP has an impact on post-natal growth, as demonstrated by the noticeably smaller pups at dosages ranging from 143 to 285 mg/kg/d (during gestation and breastfeeding) as shown by (Waterman et al., 2000) as cited in (BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022). Also consistent are the findings of two studies on developmental toxicity by (Hellwig et al., 1997) as cited in (BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022) and (Waterman et al., 1999) as cited in (BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022) after prenatal exposure to DINP with higher rates of rudimentary lumbar and/or supernumerary cervical ribs as well as negative effects on the developing kidneys of the foetus. For these developmental effects, Hellwig et al. determined a LOAEL of 1,000 mg/kg bw/d and a NOAEL of 200 mg/kg bw/d. For the effects in the study of Waterman et al. NOAEL and LOAEL values of 100 and 500 mg/kg bw/d, respectively, have been determined.

(Adamsson et al., 2009), as cited in (United States Environmental Protection Agency, 2022) and (NICNAS, 2012), showed antiandrogenic effects with signs of demasculinization with female-like nipples, small and atrophic testis and epididymal agenesis in rats being exposed from gestational day (GD) 3-17 with a NOAEL of 250 mg/kg bw/d and a LOAEL of 750 mg/kg bw/d. Impairments of sexual maturation were also observed by Boberg et al. (2011) with increased nipple retention and decreased testicular testosterone content & production from 600 mg/kg bw/d without any signs of maternal toxicity and by (Borch et al., 2004) as cited in (California EPA et al., 2013; NICNAS, 2012; United States Environmental Protection Agency, 2022) with decreased testicular testosterone content and testicular testosterone production at 750 mg/kg bw/d. Decreased anogenital distance or decreased foetal testicular testosterone were also described by Clewell et al. (2011) as cited in (NICNAS, 2012; United States Environmental Protection Agency, 2022), (Furr et al., 2014) as cited in (United States Environmental Protection Agency, 2022), Hannas et al. (2011 and 2012), (L. Li et al., 2015) or (Lee et al., 2006) as cited in (California EPA et al., 2013; NICNAS, 2012; United States Environmental Protection Agency, 2022). Testicular pathology in form of degeneration of meiotic spermatocytes and Sertoli cells were described by Masutomi et al., 2003 and 2004 as cited in (California EPA et al., 2013; United States Environmental Protection Agency 2022). No significant effects on testosterone release, luteinising hormone-stimulated testosterone, Leydig cell density and area, germ cell density or Sertoli cell proliferation were observed in a single *in vitro* study with testicular cells collected from SD rats on GD15 (Tardif et al., 2023). However, this single *in vitro* study contrasts with numerous *in vivo* studies in which clear effects were shown. In a weight-of-evidence approach, this study is therefore not considered to be of great significance.

In the most recent evaluation of the (United States Environmental Protection Agency, 2022) it was concluded that decreased body weight of pups is a sensitive indicator for developmental toxicity since the observed effects were statistically significant, dose related, increased over time of exposure and the reductions were observed in both sexes and in both F1 and F2 generations in the two-generation studies and in one-generation studies. In addition, it was argued that the effects might have long-term consequences.

The skeletal and kidney abnormalities seen in rats given DINP treatment are also considered serious by the agency since they are anatomical manifestations of disturbed development. The skeletal changes and renal consequences that were seen happened with no or little maternal toxicity. Specifically, the presence of additional cervical ribs may have major health implications. Supernumerary cervical ribs are a rare occurrence. They are not regarded as direct malformations but also not as minor variations. Their existence may suggest that a disruption in gene expression is the cause of this morphological anomaly. Furthermore, there is worry that the cervical ribs could obstruct regular blood flow and nerve activity.

It was concluded by the US EPA that the available literature provides evidence that DINP can be reasonably anticipated to cause developmental toxicity in humans.

It was considered likely that it would act via the same modes of action, including androgen deficiency. Based on (Clewell, 2011) and supported by (Hannas et al., 2011) as cited in (California EPA et al., 2013; NICNAS, 2012; United

States Environmental Protection Agency, 2022) and (Hannas et al., 2012) as cited in (California EPA et al., 2013; NICNAS, 2012); (Boberg et al., 2011) as cited in (California EPA et al., 2013; NICNAS, 2012; United States Environmental Protection Agency, 2022) a NOAEL of 50 mg/kg bw/d was proposed based on decreased T production/level and histological alterations in foetal/pup testis at a LOAEL of 250 mg/kg bw/d (ECHA, 2013).

However, for mixture risk assessment, revised points of departure (POD) for phthalates were suggested with BMDL of 10 mg/kg bw/d for DIBP, 1 mg/kg bw/d for BBP, and 5.9 mg/kg bw/d for DINP (Kortenkamp & Koch, 2020).

6 Derivation of human biomonitoring values

According to (Apel et al., 2020), the purpose of human biomonitoring (HBM) studies is to identify and quantify chemicals of concern or their metabolites in human biological material with a subsequent interpretation of the measurements to determine if (further) chemical management measures or regulation are needed. Existing data on DINP allows for the derivation of HBM guidance values (HBM-GVs) for the general population that correspond to internal exposure levels at which there is no appreciable risk.

6.1 Choice of the critical effect and POD

The choice of the critical effect should be based on prolonged exposure or exposure during a critical period of time (in case of developmental studies). There are three studies with repeated administration to rats over a period of two years in which comparable effects on the liver and kidneys occurred.

As described previously in section 5.2.3, in the first study by (Exxon Biomedical Sciences, 1986) a NOAEL of 0.03%, corresponding to 15-18 mg/kg bw/d, was established. It was based on an increased incidence of spongiosis hepatis and increased serum levels of liver transaminases in males (1.5-2x), an increase in absolute and relative liver (11-19 %) and kidney weights (5-10 %) in both sexes and a 61 % increase in relative and absolute spleen weights at 0.3 % in males. In addition, rats had enlarged livers and a minor increase in liver enzymes.

In a second study by Aristech (1994) as cited in (ECHA, 2013), a NOAEL of 0.15 %, corresponding to 88 - 109 mg/kg bw/d was established based on increased liver enzymes and increase in liver and kidney weights in both sexes including histopathological changes indicating liver toxicity. However, more liver sections in the Exxon study were examined, thus, it was considered more reliable by (ECHA, 2013).

A third study was conducted in mice and similar results were observed for liver and kidney including changes in liver and kidney weights for males and females. Regarding these effects, a NOAEL of 0.15 %, corresponding to 90 mg/kg bw/d was established. Further details on the mentioned studies with repeated exposure can be found in section 5.2.3.

According to (Apel et al., 2020), there are three options to derive the HBM-GV. The first option is to choose the POD according to the critical effect from human data, the second option is to use a defined external toxicological reference value (TRV), proposed by a European or relevant non-European institution and the third option is a POD based on a critical effect observed in experimental animal studies. Since no adequate human data is available, the second option is used. Hence, for the choice of a point of departure, toxicological reference values derived from authorities/registrants are used as listed in section 4.

In 2005, EFSA derived an individual TDI of 0.15 mg/kg bw/d for DINP, which was confirmed in 2019 (EFSA, 2005b and 2019). This TDI was based on the study by Exxon Biomedical Sciences (1986) with non-peroxisomal proliferation-related chronic hepatic and renal effects in rats leading to the NOAEL of 15 mg/kg bw/d. An uncertainty factor of 100 was applied to derive the TDI of 0.15 mg/kg bw/d. EFSA (2019) noted that DINP affects the liver at doses lower than those associated with its reproductive effects.Studies for developmental outcomes showed with a few exceptions NOAELs at higher doses than 15 mg/kg bw/d, see also Table 3-1 of US EPA 2025 (https://www.regulations.gov/document/EPA-HQ-OPPT-2018-0436-0137). The exceptions are studies from Li et al. (2015) and Lee et al. (2006). However, US EPA did not consider the study by Li et al. (2015) further as the sole study on which to derive the POD, for the following reasons “While dose-dependent increases in testes dysgenesis and decreases in fetal testicular testosterone were noted, this study had limited statistical power (n = 6). It is also unclear what the study authors considered the broad description of “testes dysgenesis” to represent, although there is some indication that they are referring to seminiferous tubule atrophy. Further, effects on male pup body weight were not dose-related, with an essentially flat dose-response across doses spanning three orders of magnitude. A similar flat dose-response was noted in the frequency distribution of cluster sizes of fetal Leydig cells, and this endpoint is of uncertain adversity. Although this study supports EPA’s conclusions regarding the endpoint for hazard identification, there is too much uncertainty in the dose-response in this study to use it quantitatively……”

Lee et al. (2006a) conducted a dietary study in which decreased male pup AGD was reported at the lowest dose tested, 40 ppm (estimated to be approximately 2 mg/kg bw/day). However, several factors reduced EPA’s

confidence in this study and its results. “First, study authors did not report dam body weight, food intake, or calculated received doses in units of mg/kg bw/day, so there is uncertainty related to the achieved doses in the study. Further, the effect of DINP on male pup AGD normalized to the cube root of bodyweight was slight (overall magnitude of effect not reported), and treatment with DBP (a more potent antiandrogen compared to DINP) at equivalent or higher doses had no effect on male pup AGD normalized to the cube root of body weight. This calls into question the significances of the slight change in AGD observed for DINP. Given these uncertainties, EPA does not consider the study by Lee et al. (2006a) suitable for use as the acute POD”.

The US EPA used a POD of 15 mg/kg bw/day to derive a MOE, as did the EFSA to derive the TDI. Both PODs refer to liver effects, but the value used by the US EPA was adopted from the studies by Lington et al. (1997), Bio/dynamics (1986). Using the POD, numerically equal to that used by EFSA, a MOE of similar magnitude to the TDI was derived.

The remaining toxicological reference values (TRVs) were not further considered. The California Environmental protection Agency's value of 146 µg/d with no significant risk level (NSRL) is based on excess cancer rate but it is not clear on which study it is based, nor is there any primary data on which the value of 290 µg/kg bw/d derived by Health Canada is based.

Therefore, the TDI as derived by EFSA and supported by the MOE of US EPA is preferred as TRV for the derivation of HBM-GV. It is based on the most sensitive endpoint considering all animal studies carried out. Recent research including epidemiology studies does not justify the selection of a different POD Possible differences between rats and humans regarding bioavailability of DINP and its metabolites are covered by the default factor 10 for interspecies differences in the TDI derivation. An additional factor, as proposed by ECHA (2013), is not considered necessary (see also information in Table VI. 5).

6.2 Derivation of HBM-GVs

If specific biomarkers for excretion are available, an HBM-GV can be derived from a TRV by using the urinary mass balance approach (see also Apel et al., 2020). There are two possibilities: the calculation based on an individual major specific metabolite (Equation 4), or on the sum of most relevant specific metabolites detected in the urine (example, see Equation 5).

The excretion in relation to the administered dose on a molar basis was for the most important specific metabolites 12.3 % for 7-MHINP (OH-MINP) and 10.9 % for 7-MCIOP (cx-MINP), respectively (Anderson et al., 2011). The resulting urinary excretion factors (F_{ue}) are the decimals of the percentages resulting in values of 0.123 for OH-MINP and 0.109 for cx-MINP as inserted into the formula.

The molecular weight of the parent substance DINP is 418.62 g/mol, of OH-MINP is 308.37 g/mol and of cx-MINP is 336.4 g/mol. In addition, as the TRV is given in relation to body weight, the daily urine is adjusted to body weight: The current default for women is 0.035 L/(kg bw x d)[20], and for children and men is 0.03 L/(kg bw x d)[21].

The calculations shown below were carried out using these values.

Equation 4: Urinary mass balance approach with one metabolite

$$HBM - GV = TRV \times \frac{\left(\frac{MW(Metabolite) \times Fue(Metabolite)}{MW(Substance)}\right)}{Daily\ urinary\ flow\ rate}$$

Equation 5: Urinary mass balance approach with two metabolites

$$HBM - GV = TRV \times \frac{\left(\frac{MW(Met.1) \times Fue(Met1) + MW(Met.2) \times Fue(Met.2)}{MW(Substance)}\right)}{Daily\ urinary\ flow\ rate}$$

[20] German HBM Commission, 2025, submitted
[21] German HBM Commission, 2025, submitted

Equation 6: Urinary mass balance approach for the major specific metabolite OH-MINP, for children and men

$$HBM-GV = 0.15\frac{\frac{mg}{kg\ bw}}{d} \times \frac{\left(\frac{308.37\frac{g}{mol} \times 0.123}{418.62\frac{g}{mol}}\right)}{\frac{0.03\frac{L}{kg\ bw}}{d}} = 0.453\frac{mg}{L}, rounded\ 0.45\ \frac{mg}{L}$$

For women, the corresponding HBM-GV with the currently valid default value for the daily urinary flow rate of 0.035 L/kg bw/d is as follows: 0.388 mg/L

Equation 4: Urinary mass balance approach for the major specific metabolite cx-MINP, for children and men

$$HBM-GV = 0.15\frac{\frac{mg}{kg\ bw}}{d} \times \frac{\left(\frac{336.4\frac{g}{mol} \times 0.109}{418.62\frac{g}{mol}}\right)}{\frac{0.03\frac{L}{kg\ bw}}{d}} = 0.438\frac{mg}{L}, rounded\ 0.44\ \frac{mg}{L}$$

For women, the corresponding HBM-GV with the currently valid default value for the daily urinary flow rate of 0.035 L/kg bw/d is as follows: 0.375 mg/L

Equation 5: Urinary mass balance approach for the two specific metabolites OH-MINP and carboxy-MINP, for children and men

$$HBM-GV = 0.15\ \frac{\frac{mg}{kg\ bw}}{d} \times \frac{\left(\frac{308.37\frac{g}{mol}\ \times\ 0.123 + 336.4\frac{g}{mol} \times 0.109}{418.62\frac{g}{mol}}\right)}{\frac{0.03\frac{L}{kg\ bw}}{d}} = 0.891\ \frac{mg}{L}$$

For women, the corresponding HBM-GV with the currently valid default value for the daily urinary flow rate of 0.035 L/kg bw/d is as follows: 0.763 mg/L, rounded 0.76 mg/L

When calculating for individual major specific metabolites, the formula results for OH-MINP in a rounded HBM-GV of 0.45 mg/L urine for children and men and 0.39 mg/L urine for women. For cx-MINP the rounded HBM-GV is 0.44 mg/L urine for children and men and 0.38 mg/L urine for women. The rounded HBM-GV for the sum of both metabolites is 0.89 mg/L urine for children and men and 0.76 mg/L mg/L for women.

The derived HBM-GVs represent the concentration in urine for the respective metabolites up to which - according to current knowledge and for single substance evaluation - there is no risk of adverse health effects.

6.3 However, when assessing HBM results, it must be taken into account that exposure to mixtures of substances is usually present, so that the additional use of a MAF (mixture assessment factor/mixture allocation factor) or another suitable methodology should be considered for further assessments. At least structurally related substances or substances that display the same effect targets should be considered together for the next review and refinement of the HBM-GV.Level of confidence attributed to the derived HBM-GVs

As mentioned in the HBM4EU concept document to derive HBM-GVs, it is suggested to attribute a level of confidence (low, medium or high) to each calculated HBM-GV. The level of confidence should reflect the uncertainties identified during the elaboration of the value and could constitute a good incentive to later revise values with an estimated 'lower' level of confidence.

The overall data base for toxicokinetics is limited. The data used for calculation of urinary excretion fraction was derived from a study with ten male and female volunteers, who received a single oral DINP dose of 0.012 mg/kg bw and 0.121 mg/kg bw/d in two study periods (Anderson et al., 2011). As toxicokinetics are subject to considerable individual variability, it is difficult to draw general conclusions from this individual measurement. The variability can depend in particular on the age, gender or ethnicity of the test person but can also vary in supposedly similar population groups. Hence, the data is not representative of the entire population. However, since the TDI used as POD already includes a safety factor of 10 to cover intraspecies variability, it can be concluded that the variability that has occurred is already covered to a certain extent. Moreover, the results obtained from the study by Anderson et al. (2011) are partly confirmed by the study of Koch and Angerer (2007), see section 5.1.1. The deviations between the mean values of Anderson et al. (2011) and the respective values of Koch and Angerer (2007) are consistently less than a factor of 2. Therefore, the level of confidence is **high/medium**.

Another aspect to consider is the applicability of the urinary mass balance approach. For DINP, a maximum of 43.6 % of the administered dose was recovered in the urine, depending on the study. Animal studies show that the administered dose is almost completely excreted via urine and feces within 48 hours. Therefore, it can be assumed that no bioaccumulation takes place and biomarker concentration in urine can be considered directly proportional to the amount absorbed.

The level of confidence in the choice of the EFSA TDI with 0.15 mg/kg bw/d as the critical dose is **medium**. The TDI is based on a chronic toxicity study in rats according to OECD 452 (Exxon Biomedical Sciences, 1986) with a NOAEL of 15-18 mg/kg bw/d for liver effects in males and females and an assessment factor of 100 (to account for inter- and intraspecies variability). The same study was identified as the key study in the performed literature search for this project. Hence, the POD is considered suitable. The US EPA estimates a MOE value of similar magnitude to the TDI value based on a POD of the same magnitude as that used by EFSA, but from another study in which liver effects are also the critical effects. According to current data, the TDI is also protective with regard to endocrine and carcinogenic effects. Nevertheless, the key study for the derivation of the TDI is based on animal data. The best case for deriving HBM-GVs would have been the availability of human data.

The overall level of confidence is considered to be **medium**. The limited availability of toxicokinetic data, which might be subject of considerable variation, is already covered to a certain extent by the TDI as mentioned before. Therefore, an extrapolation to other population groups should be possible without the introduction of substantial uncertainty.

It should be noted, though, that the levels of confidence suggested in this document are based on expert judgement regarding the accuracy of the data and the process of calculation used to derive the HBM guidance values, rather than being established in accordance with a recognized and accepted methodology.

6.4 Note regarding the necessary specificity of the analytical method used

According to Norway's (NIPH) comment, it is known that in some analysis methods, OH-MINP might have an interference from a carboxyl metabolite of terephthalate if they are not separated. And this may give wrong results

for urinary concentrations of OH-MINP. This means that those conducting HBM studies must ensure that their method is appropriate to measure OH-MINP correctly. Otherwise, the focus should be on cx-MINP.

6.5 Note regarding an HBM-GV for workers

Under HBM4EU a provisional HBM-GV for workers has been derived for DINP (D5.5 – Human biomonitoring in risk assessment: 2nd set examples on the use of HBM in risk assessments of HBM4EU priority chemicals). The same critical effects as presented above and standard assumptions and assessment factors as used by ECHA previously for the derivation of occupational DNELs for DBP, BBP and DEHP had been used. Thus, the calculation for deriving a human equivalent point of departure (POD) was based on the same NOAEL of 15 mg/kg bw/d from an animal study. It was multiplied with the following factors to correct for several differences:

- 1/0.38 for the standard respiratory volume in m^3/kg bw per day
- 50/75 for the 50% oral absorption in rats and assumed 75% inhalation absorption in adult humans
- 6.7/10 to account for increased breathing volume (m^3) from rest to light work
- 7/5 for a shorter exposure duration in the occupational vs. general population (7 vs. 5 days a week)

The resulting human equivalent POD (25 mg/m^3) was further corrected with the assessment factors of 2.5 (for interspecies differences in toxicodynamics) and 5 (for intraspecies variation, which is assumed smaller in the occupational vs. the general population), resulting in an occupational inhalation DNEL of 2.0 mg/m^3. The corresponding inhaled dose as mg/kg was calculated by multiplying the inhalation DNEL with a factor of 10 (to account for the average breathing volume of 10 m^3 during a working day), and dividing it by 70 (assumed average body weight of workers), resulting in 0.28 mg/kg (8 h TWA).

The further calculation using the urinary mass balance approach was performed with slightly different input data compared to the calculation of the HBM-GVs for the general population, in particular the Fue refers to 24 h instead of 48 h and the urine volume used was 1.5 L for a 70 kg person. The values derived under HBM4EU should therefore be recalculated and will be presented for discussion in year 4 of PARC. The extent to which absorption as a gas or by means of dust plays a role for the workers must also be discussed here.

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8 Appendix – Toxicological profile

8.1 Search Terms

Scopus:

TITLE-ABS-KEY ((dinp OR {Diisononyl phthalate} OR {Di-isononyl phthalate} OR {MINP} OR {Monoisononyl-phthalate} OR {Monoisononyl phthalate} OR {Mono-isononyl-phthalate} OR {Mono-isononyl phthalate} OR {7OH-MMeOP} OR {Mono-(4-methyl-7-hydroxyoctyl)-phthalate} OR {Mono-(4-methyl-7-hydroxyoctyl) phthalate} OR {Mono(4-methyl-7-hydroxyoctyl)-phthalate} OR {Mono(4-methyl-7-hydroxyoctyl) phthalate} OR {7oxo-MMeOP} OR {Mono-(4-methyl-7-oxooctyl)-phthalate} OR {Mono-(4-methyl-7-oxooctyl) phthalate} OR {Mono(4-methyl-7-oxooctyl)-phthalate} OR {Mono(4-methyl-7-oxooctyl) phthalate} OR {7cx-MMeHP} OR {Mono-(4-methyl-7-carboxyheptyl)-phthalate} OR {Mono-(4-methyl-7-carboxyheptyl) phthalate} OR {Mono(4-methyl-7-carboxyheptyl)-phthalate} OR {Mono(4-methyl-7-carboxyheptyl) phthalate}) AND (health OR adverse OR {mode of action} OR toxic* OR neurotox* OR epidem* OR cohort OR genotox* OR genetic* OR carc* OR cancer OR reproduc* OR reprotox* OR developmental OR fertil* OR disease OR neurologic* OR metabol* OR adme OR pbpk OR pbtk OR excret* OR {in vivo} OR {in vitro} OR {in silico} OR endocrine OR {half-life} OR immun* OR moa))

Pubmed:

(DINP[Title/Abstract] OR "Diisononyl phthalate"[Title/Abstract] OR "Di-isononyl phthalate"[Title/Abstract] OR "28553-12-0"[Title/Abstract] OR MINP[Title/Abstract] OR "Monoisononyl-phthalate"[Title/Abstract] OR "Monoisononyl phthalate"[Title/Abstract] OR "Mono-isononyl-phthalate"[Title/Abstract] OR "Mono-isononyl phthalate"[Title/Abstract] OR "7OH-MMeOP"[Title/Abstract] OR "Mono-(4-methyl-7-hydroxyoctyl)-phthalate"[Title/Abstract] OR "Mono-(4-methyl-7-hydroxyoctyl) phthalate"[Title/Abstract] OR "Mono(4-methyl-7-hydroxyoctyl)-phthalate"[Title/Abstract] OR "Mono(4-methyl-7-hydroxyoctyl) phthalate"[Title/Abstract] OR "7oxo-MMeOP"[Title/Abstract] OR "Mono-(4-methyl-7-oxooctyl)-phthalate"[Title/Abstract] OR "Mono-(4-methyl-7-oxooctyl) phthalate"[Title/Abstract] OR "Mono(4-methyl-7-oxooctyl)-phthalate"[Title/Abstract] OR "Mono(4-methyl-7-oxooctyl) phthalate"[Title/Abstract] OR "7cx-MMeHP"[Title/Abstract] OR "Mono-(4-methyl-7-carboxyheptyl)-phthalate"[Title/Abstract] OR "Mono-(4-methyl-7- carboxyheptyl) phthalate"[Title/Abstract] OR "Mono(4-methyl-7- carboxyheptyl)-phthalate"[Title/Abstract] OR "Mono(4-methyl-7- carboxyheptyl) phthalate"[Title/Abstract]) AND (health[Title/Abstract] OR adverse[Title/Abstract] OR "mode of action"[Title/Abstract] OR toxic*[Title/Abstract] OR neurotox*[Title/Abstract] OR epidem*[Title/Abstract] OR cohort[Title/Abstract] OR genotox*[Title/Abstract] OR genetic*[Title/Abstract] OR carc*[Title/Abstract] OR cancer[Title/Abstract] OR reproduc*[Title/Abstract] OR reprotox*[Title/Abstract] OR developmental[Title/Abstract] OR fertil*[Title/Abstract] OR disease[Title/Abstract] OR neurologic*[Title/Abstract] OR metabol*[Title/Abstract] OR ADME[Title/Abstract] OR PBPK[Title/Abstract] OR PBTK[Title/Abstract] OR excret*[Title/Abstract] OR "in vivo"[Title/Abstract] OR "in vitro"[Title/Abstract] OR "in silico"[Title/Abstract] OR endocrine[Title/Abstract] OR "half-life"[Title/Abstract] OR immun*[Title/Abstract] OR MOA[Title/Abstract])

8.2 Findings in humans

Table VI.35: Overview of toxicokinetics and metabolism in humans

Reference	Cited in	Findings
Anderson et al. 2011	(California EPA et al., 2013; United States Environmental Protection Agency, 2022)	• Ten male and ten female subjects were administered in a single oral dose 0.012 mg/kg bw deuterated DINP (D4-DINP) and 0.121 mg/kg bw in two study periods. • More than 90 % of the metabolites were excreted in the first 24 hours of urine collection, the remainder in the second 24 hours. Total metabolites were 32.9±6.4 % of the administered dose. Fractional excretion of primary and secondary metabolites was 3.1%, 6.6%, 12.3% and 10.9% for MINP, 7-MOINP, 7-MHINP and 7-MCIOP, respectively. The estimated elimination half-lives of the metabolites were between four and eight hours.
Becker et al. 2009	(California EPA et al., 2013)	• Investigation of DINP metabolites in urine samples from 599 children aged 3 to 14 years from Germany collected between May 2007 and February 2008 • The median urine concentrations of MHINP, MOINP and MCIOP in children were 11.0, 5.4 and 12.7 µg/L, respectively. The concentrations of these metabolites were significantly higher in young children (3-5 years) than in children in older age groups (6-8 years; 9-11 years; 12-14 years).
(Schwedler et al., 2020)		• 2256 first-morning void urine samples of 3-17 years old children and adolescents in Germany were investigated for DINP among others. • Metabolites of DINP were found in 97-100% of the participants (2249 samples with OH-MINP, 2256 samples with oxo-MINP and 2250 samples with cx-MINP) • 0.007% of the participants exceeded health-based guidance values for DINP in the current GerES V. • Sums of DiBP, DnBP, DEHP DiNP and DiDP urinary metabolite levels, constantly decreased with age, being highest for the 3–5 years old and lowest for the 14–17 years old participants. • Gender-specific differences with girls having higher geometric means of DINP metabolites • Levels of DINP declined with increasing socioeconomic status. • Migration background was associated with higher GMs of DINP metabolite concentrations.
(Lange et al., 2022)		• HBM4EU assessed human biomonitoring data on five reprotoxic phthalates (DEHP, DiBP, DnBP, BBzP, DiNP) in urine samples of 4198 children aged 6-11 years and adolescents aged 12-18 years from 12 countries, which were sampled between 2014 and 2021. • DnBP and DiBP were the clear drivers of the mixture risk. • DiBP and DnBP contributed mostly to the hazard index explaining more than 90% of the HI, whereas the contribution of DEHP and DiNP are negligible with 2-4 % for DiNP.
(Vogel et al., 2023)		• Exposure data of 5600 children (6-11 years) and adolescents (12-18) from up to 12 countries covering North, East, South and West European regions were assessed.

Reference	Cited in	Findings
		• Exposure to Phthalates and DINCH was deduced from urinary excretions of metabolites. • For DINP OH-MINP and cx-MINP were used as biomarkers. • The sum of both DINP metabolites was nine times higher in France than in Belgium.
Calafat et al. 2011	(California EPA et al., 2013)	• Assessment of DINP exposure in persons aged ≥ 6 years in the general US population using data from the 2005-2006 National Health and Nutrition Examination Survey (NHANES). • MCIOP was present in 89.9 per cent of those studied, with a mean concentration of 5.1 µg/L. The results of the NHANES survey also indicate that MCIOP concentrations are inversely related to the age of the study participants, with higher concentrations in children than in adults.
Koch and Angerer 2007	(California EPA et al., 2013; NICNAS, 2012; United States Environmental Protection Agency, 2022)	• A 63-year-old man was orally administered 1.27 mg/kg deuterium (D4)-labelled DINP in bread. • 40 and 44% were excreted renally after 24 and 48 h, respectively. • The major metabolites extracted in the urine were: MHINP (ca. 20% of the administered dose), MOINP (ca. 10%), MCIOP (ca. 10%) and MINO (ca. 2%). However, due to differences in elimination kinetics, 5.9 % of MOINP, 8.8 % of MHINP and 15 % of MCIOP were excreted on the second day after administration. These four metabolites accounted for 43.6 % of the oral DINP dose in urine within 48 hours after administration.
Koch et al. 2011a	(California EPA et al., 2013)	• DINP exposure of German children aged 5.6 to 6.7 years was evaluated via metabolite concentrations in urine samples collected in February - March 2007. • 96, 78 and 77 % of the samples contained detectable concentrations of MHINP, MOINP and MCIOP, respectively. The median concentrations of MHINP, MOINP and MCIOP were 7.0, 4.2 and 13.4 µg/L, respectively. The 95th percentile of the concentrations of MHINP, MOINP and MCIOP were 25.5, 12.5 and 45.5 µg/L, respectively.
Koch et al. 2011b	(California EPA et al., 2013)	• Of five workers in automobile production who sealed seams with DINP-based plastisol, urine samples were collected before and after the shifts. Urine samples were collected from 10 other workers at the same plant for comparison. • The DINP exposure levels of the comparison group were similar to those of the general population, but the post-shift DINP exposure levels were higher than those of the comparison group for all plastisol workers.
Lin et al. 2011	(California EPA et al., 2013)	• Phthalate metabolites were analysed in urine, umbilical cord serum and breast milk of Taiwanese women after delivery in the period December 2001 - November 2002. • Phthalate metabolites were also measured in the urine of the children who were newborns at the beginning of the study, in the age groups 2 - 3 years and 5 - 6 years. • The DINP metabolites measured were MHINP, MOINP and MCIOP. The proportion of DINP metabolites in the total pool of phthalate metabolites was higher in the urine of children (4.39

Reference	Cited in	Findings
		and 8.31 % at ages 2 to 3 and 5 to 6 years, respectively) than in mothers (0.83 %).
Silva et al. 2004	(ECHA registration dossier, 2022)	• Infrequent detection of MINP in urine samples
Silva et al. 2006b	(California EPA et al., 2013)	• The urine concentrations of MINP and the oxidative MINP metabolites mono(hydroxyisononyl)phthalate (MHINP), mono(oxoisononyl)phthalate (MOINP) and mono(carboxyisooctyl)phthalate (MCIOP) were measured in 129 adults with no known occupational or other exposure to DINP. • MHINP, MOINP and MCIOP were detected in 100, 97 and 87 % of the samples, respectively, with geometric means of 11.4, 1.2 and 8.6 µg/L, respectively. MINP concentrations were below the detection limit.
Zeman et al. 2013	(California EPA et al., 2013)	• Phthalate exposure of a group of pregnant French women was assessed in October 2007. A total of 279 urine samples were collected. • Mean and median urinary MINP concentrations were below the limit of quantification (4 µg/L). Median urinary MHINP, MOINP and MCIOP concentrations were 7.6, 6.1 and 16.0 µg/L, respectively. The median urinary MHINP, MOINP and MCIOP concentrations were 3.1, 2.2, and 6.1 µg/L, respectively. The authors estimated the daily median and 95th percentile of daily dietary DINP intake to be 1.1 and 7.6 µg/kg-day, respectively, based on the median combined urinary metabolite concentrations.

In the following, results on the absorption of DINP after oral exposure are summarized (Table VI.36), as well as metabolites that could be detected in different species after exposure. This information was taken from Environment Canada and Health Canada 2015.

Table VI.36: Oral absorption percentages for DINP as taken from (Environment Canada and Health Canada 2015)

Reference	Species	Dose	Basis	Excretion (% of dose)
Anderson et al. 2011	Human	0.78 and 7.3 mg	Urine	33 ± 6.4 % in 48 h
Koch and Angerer 2007	Human	1.28 mg/kg	Urine	44 % over 48 h
McKee et al. 2002	Rat	50 mg/kg 500 mg/kg 50-500 mg/kg 50, 150, 500 mg/kg daily	Urine Urine Urine + bile (estimate) Urine Urine Urine + bile (estimate)	49 % in 72 h 39 % in 72 h 75 % in 72 h 56-62 % in 24 h 62-64 % in 72 h 90 % in 72 h

8.3 Animal findings

Table VI.37: Overview of toxicokinetics and metabolism in animals

Reference	Cited in	Findings
Clewell et al. 2013	(Environment Canada and Health Canada, 2015)	• DINP was administered by gavage to pregnant rats at concentrations up to 750 mg/kg body weight/day during GD 12-19. • In urine, MCIOP was the major metabolite, while MINP and its glucuronidated form (MINP-Gluc) were almost negligible. • In contrast, all metabolites were present in maternal plasma (MINP in similar concentrations to MCIOP). It was concluded that urinary excretion of MINP and MINP-Gluc is limited and that these urinary metabolites are poor predictors of plasma and tissue disposition of DINP. • MINP and its oxidative metabolites as well as its glucuronidated form were all present in foetal plasma, testis and amniotic fluid. MINP-Gluc was the only metabolite present in higher concentrations in the foetal plasma than in the maternal plasma, indicating a possible placental transfer of this form or, more likely, that conjugation could take place in the foetus.
Silva et al. 2006	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	• A single dose of 300 mg/kg body weight DINP was administered to pregnant rats and the oxidative metabolites were determined in the urine. • MCIOP was the most important metabolite found in the urine, while MINP and DINP were not detectable in significant amounts.
Hazleton 1972	(NICNAS, 2012; NTP, 2003)	• Male albino mice exposed orally to 2500 mg/kg bw/d for six days • Within 72h about 85% excreted in faeces, 12% in urine, most within 72h
McKee et al. 2002	(Environment Canada and Health Canada, 2015; NICNAS, 2012; United States Environmental Protection Agency, 2022).	• Male and female rats, ^{14}C-DINP was administered orally (50 or 500 mg/kg body weight/day for one day or 50, 150 or 500 mg/kg body weight/day for five consecutive days). • After single exposure, absorption was found to decrease with increasing dose (49% of the low dose of 50 mg/kg bw/d and 39% of the high dose of 500 mg/kg bw/d were excreted in urine within 72 hours, while the proportion in faeces increased slightly (50% and 52% of the low and high doses, respectively). • Oxidative metabolites and phthalic acid were the main metabolites detected in the urine in this study and the authors found that of the administered dose, about 40-50% was excreted in the urine of rats, while the remaining about 40-50% was recovered in faeces (as DINP and MINP). • About 66% of administered dose excreted in urine within 72h following repeated exposure

Reference	Cited in	Findings
Midwest Research Institute 1983a	(NTP, 2003)	• Dermal exposure to ^{14}C-DINP was studied in male Fischer 344 rats: • amount absorbed after 7 days ranged from 2 to 4% of the dose. • 93–99% of the administered radioactivity was recovered at the site of application. • Radioactivity in faeces and gut of the exposed rats suggested some excretion via the biliary route.
Midwest Research Institute 1983b	(NTP, 2003)	• ^{14}C collected in the urine of rats following a single oral dose of ^{14}C-DINP was in the form of phthalic acid or side-chain oxidation products of the monoester (MINP), while monoester itself, as well as the diester, was present in only trace amounts. • The relative amount of phthalic acid in the urine decreased at the high dose • In faeces, 8 and 41% of the radioactivity was associated with the diester following administration of a low (50 mg/kg) or a high (500 mg/kg) oral dose of ^{14}C-DINP. This indicates saturation of metabolism at the high dose. The remainder of the faecal radioactivity was associated with the monoester or its side-chain oxidation products. • Major metabolites in the liver were the monoester and its side-chain oxidation products. The same metabolites and phthalic acid were in testes. • Fat contained the monoester and its oxidation products. Repeated exposures revealed similar metabolites in the tissues. • In male and female Fischer 344 rats receiving single or repeated oral doses of ^{14}C-DINP, radioactivity also cleared from the tissues rapidly, but analysis of tissues soon (within 1 hour) after the exposure indicated that the highest levels were in liver (4.7% of administered dose), kidneys (0.31%), and blood (1.62 %). • No bioaccumulation was observed 72 hours postdosing.
Unnamed 1972	(ECHA registration dossier, 2022)	• Male albino rats received "cold" C14 labelled DINP daily for five days. On the sixth day, each animal received a single dose of "hot" compound (about 2,500 mg/kg/d) and placed in metabolism cages for 72 hours. • The amount of compound excreted in the urine within 72 hours ranged from 8% to 18.34% of the administered dose. The majority of the radioactivity found was excreted in the first collection (12 hours). Thereafter, the amount of radioactivity found fell off rapidly so that by the 72-hour collection period less than 0.1% of the dose was found in all animals except in one animal. • Over 80% of the administered dose was excreted in the faeces and most of the radioactivity was excreted within 24-hours after dosing. Between the 60- and 72-hour post dosage period no animal excreted over 0.2% of the administered dose.

Reference	Cited in	Findings
		• No bioaccumulation potential based on study results
(Hou et al. 2013)		• DINP was injected i.v. at 10 mg/kg or given orally at 100 mg/kg to rats. • Urine and feces were collected at 0–12, 12–24 and 24–48 h post dosing. About 200 µL of blood samples was withdrawn at 0, 5, 15, 30, 45, 60, 90, 120, 240, 360 and 480 min. • Elimination half-life was 364 ± 146 min for i.v. and 150 ± 58 min after oral exposure. • Oral bioavailability was low with about 1.19%. • Total excretion of DINP up to 48 hours was 13.64 ± 3.99 % in the faeces.

Table VI.38: Overview of acute toxicity studies

Reference	Cited in	Route of Exposure	Species	Results (LD50/LC50)	
BASF 1981a	(NICNAS, 2012)	Oral	Rat	>10,000 mg/kg bw	
Hazleton 1968c	(NICNAS, 2012)	Oral	Rat	>10,000 mg/kg bw	
Hazleton 1980b	(NICNAS, 2012)	Oral	Rat	>50,000 mg/kg bw	
Hüls 1985a	(NICNAS, 2012)	Oral	Rat	>10,000 mg/kg bw	
Krauskopf 1973	(United States Environmental Protection Agency, 2022)	Oral	Rat	>10,000 mg/kg bw	
Midwest Research Institute 1981	(NICNAS, 2012)	Oral	Rat	>40,000 mg/kg bw	
Hazleton 1968a	(NICNAS, 2012)	Dermal	Rabbit		>3,160 mg/kg bw
Hazleton 1980a	(NICNAS, 2012; United States Environmental Protection Agency, 2022)	Inhalation	Rat		>4.4 mg/L
Unnamed 1981	(ECHA registration dossier, 2022)	Inhalation	Rat		> 0.07 mg/L

Table VI.39: Overview of animal studies with repeated exposure.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Aristech 1994, Moore 1998a Covance 1998, Moore 1998, Butala et al. 1996	(California EPA et al., 2013; ECHA, 2013; Environment Canada and Health Canada, 2015; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	F344 rats (OECD 452)	0, 0.05, 0.15, 0.6 and 1.2 % (0, 29, 88, 359, 733 mg/kg bw/ for males, 0, 36, 109, 442, 885 mg/kg bw/d for females)	Diet, 2 years, DINP-1 (68515-48-0)	NOAEL: 0.15 % (88-103 mg/kg bw/d)	Increased incidence of spongiosis hepatis in males from 0.6 %, increased serum levels of liver transaminases, abs. and rel. liver and kidney weights, and histopathological findings indicating liver toxicity in m and f from 0.6 %
Barber et al. 1987	(Environment Canada and Health Canada, 2015) (United States Environmental Protection Agency, 2022)	Fisher 344 rats	0, 639, 1192, 2195 (males); 0, 607, 1198, 2289 (females) (estimated from 0, 0.6, 1.2, 2.5%).	Diet, 3 weeks; DINP-1 (68515-48-0)	LOAEL: 607-639	Increase in absolute and relative liver weight with increased 11- and 12-hydroxylase activity, hypolipidaemic effects, cytoplasmic basophilia in hepatocytes at 1.2% and eosinophilia each at the highest dose.
BASF AG 1987	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Wistar rats	0, 333, 1101, 3074 on day 7; 0, 152, 512, 1543 on day 91 (male); 0, 379, 1214, 3224 on day 7; 0, 200, 666, 2049 on day 91 (female) (estimated from 0, 3000, 10 000, 30 000 ppm).	Diet, 13 weeks; DINP-2 (28553-12-0)	LOAEL: 152-200	Clinical chemistry and liver changes associated with hepatotoxicity (tendency to decreased triglyceride levels and decreased peripheral fat deposition in hepatocytes).
BIBRA 1986	(NTP, 2003; United States Environmental Protection Agency, 2022)	Fischer 344 rats male/female)	(approximately 639, 1,192, 2,195 mg/kg/day in males; and 607, 1,193, 2,289 mg/kg/day in females	Diet, 21 days	Not specified	Significant decrease in mean body weight and food intake (male and female); significant dose-dependent increase in absolute and relative liver weight (male and female); mean relative kidney weight increased (male and female)

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Bio/dynamics 1982a	(Environment Canada and Health Canada, 2015; NICNAS, 2012; United States Environmental Protection Agency, 2022)	Fischer 344 rats (male/female)	77, 227, 460, 767, 1554 (estimated from 0, 0.1, 0.3, 0.6, 1.0, 2.0%)	Diet, 13 weeks; DINP-1 (68515-48-0)	NOAEL: 77 LOAEL: 227	Increase in kidney and liver weight accompanied by histopathological changes in the liver at the two highest doses and in the kidneys from 0.6%.
Bio/dynamics 1982b	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Fisher 344 rats	0, 201, 690 (males); 0, 251, 880 (females) (estimated from 0, 0.3, 1.0%).	Diet, 13 weeks; DINP-1 (68515-48-0)	LOEL: 201-251	Increase in relative kidney and liver weight accompanied by a dose-dependent decrease in triglycerides and altered urine chemistry.
Bio/dynamics 1982c	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Fischer 344 rats (male)	0, 1700 (estimated from 0, 2%)	Diet, 7 days; DINP-1 (68515-48-0)	LOAEL: 1700	Increased liver and kidney weight with mild liver congestion, accompanied by a mild to moderate decrease in cholesterol, a mild to severe decrease in triglycerides, and a mild decrease in alkaline phosphatase.
Liang and Yan 2020		42 SPF Balb/c mice (male)	0.02, 0.2, 2, 20, and 200	Dermal exposure, 28 days, DINP (68515-48-0)	Not specified	Slight dermal changes at 0.02, 0.2 and 2 mg/kg, significant dermal changes at 20 and 200 mg/kg. Significant increase in liver-to-bw ratios in both groups. Increased kidney-to-bw ratio in the HD group. Increased liver damage with increased dosage. Higher DINP levels led to lower GHS levels.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Exxon 1986, Lington et al. 1997	(BAuA, 2001; California EPA et al., 2013; ECHA, 2013; Environment Canada and Health Canada, 2015; Environment Canada Health Canada, 2020; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	F344 rats (OECD 452)	0, 0.03, 0.3 and 0.6 % (0, 15, 152, 307 mg/kg bw/d for males, 0, 18, 184, 375 mg/kg bw/d for females)	Diet, 2 years, DINP-1 (68515-48-0)	NOAEL: 0.03% (15-18 mg/kg bw/d)	Increased incidence of spongiosis hepatitis, serum levels of liver transaminases, abs. and rel. spleen weights in males from 0.3 %, increased absolute and relative liver and kidney weights and histopathological findings indicating liver toxicity in m and f from 0.3 %
Hall et al. 1999	(BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	Marmoset (male/female)	0, 100, 500, or 2,500	Gavage, 13 weeks	NOAEL: 500	Bw and bw gain decreased at 2,500 mg/kg bw/d
Hazleton 1969	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	New Zealand White rabbit	0, 500, 2500 (estimated from 0, 0.5, 2.5 ml/kg)	Dermal, 6 weeks (24 h on intact and abraded skin, 5 times per week); DINP-1 (68515-48-0)	NOAEL: 2500 (Environment Canada and Health Canada, 2015) NOAEL: 500 (NICNAS, 2012)	Slight or moderate erythema, and slight desquamation at 2500 mg/kg bw/d (NICNAS, 2012)
Hazleton 1981a	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Sprague-Dawley rats	0, 60, 180, 600 (estimated from 0, 1000, 3000, 10 000 ppm)	Diet, 13 weeks; DINP-2 (28553-12-0)	NOAEL: 600 (female) LOAEL: 60 (male), 180 (female)	Increase in renal lesions (males), increase in relative liver weight (females).

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Hazleton 1991a	(ECHA registration dossier, 2022; Environment Canada and Health Canada, 2015; NICNAS, 2012; United States Environmental Protection Agency, 2022)	Fisher 344 rats	0, 176, 354, 719, 1545 (male); 0, 218, 438, 823, 1687 (female).	Diet, 13 weeks; DINP-2 (28553-12-0)	LOAEL: 176-218	Increase in kidney and liver weight accompanied by histopathological changes in the liver at the highest doses and in the kidneys from 5000 ppm.
Hazleton 1991b	(ECHA registration dossier, 2022; Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	B6C3F1 Mice	0, 635, 1377, 2689, 6518 (male); 0, 780, 1761, 3287, 6920 (female) (estimated 0, 3000, 6000, 12 500, 25 000 ppm).	Diet, 4 weeks; DINP-2 (28553-12-0)	LOAEL: 635-780	Histopathological changes in the liver at all doses tested
Hazleton 1992	(Environment Canada and Health Canada, 2015; NICNAS, 2012; United States Environmental Protection Agency, 2022)	B6C3F1 Mice	0, 365, 972, 2600, 5770 (estimated from 0, 1500, 4000, 10 000, 20 000 ppm)	Diet, 13 weeks; DINP-2 (28553-12-0)	NOAEL: 365 LOAEL: 972	Increase in absolute and relative liver weight accompanied by liver enlargement and histopathological changes at the two highest doses tested, decreased absolute epididymis and testes weight.
Hazleton Laboratories 1971a	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Rats	0, 50, 150, 500	Diet, 3 months; DINP-2 (28553-12-0)	NOEL: 150 LOEL: 500	Increased kidney weight, liver weight and hepatocyte hypertrophy

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Hazleton Laboratories 1971b	(Environment Canada and Health Canada, 2015; NICNAS, 2012; United States Environmental Protection Agency, 2022)	Dog	0, 37, 160, 2000 (estimated from 0, 0.125, 0.5, 2.0%)	Diet, 13 weeks; DINP-1 (68515-48-0), DINP-2 (28553-12-0)	NOAEL: 37 (male), 160 (female) LOAEL: 160 (male), 2000 (female)	Increase in absolute and relative liver weight accompanied by histopathological changes at the highest dose tested (males), increase in absolute and relative liver and kidney weight accompanied by histopathological changes in both organs (females).
Huls AG 1992	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Fischer 344 rats (female)	0, 25, 75, 150, 1500	Gavage, 14 days; DINP-1 (68515-48-0), DINP-2 (28553-12-0), DINP-3 (28553-12-0)	LOEL: 25 - 75	Increased activity of DOS (dodecanoic acid 12-hydroxylase) at 25 mg/kg bw/d for DINP-1 and at 75 mg/kg bw/d for DINP-2 and DINP-3
Huntingdon Life Sciences 1998	(Environment Canada and Health Canada, 2015)	Marmoset Monkeys	0, 100, 500, 2500	Gavage, 13 cots; DINP-2 (28553-12-0)	NOAEL: 500 LOAEL: 2500	Decrease in body weight and increase in body weight
Kaufmann et al. 2002	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	B6C3F1 Mice	0, 117, 350, 913, 1860 (males); 0, 167, 546, 1272, 2806 (females) (discounted from 0, 500, 1500, 4000, 8000 ppm).	Diet; 1 or 4 weeks; DINP-1 (68515-48-0)	NOEL: 117-167 LOEL: 350-546	Increase in liver weight, peroxisomal volume and peroxisomal enzyme activity
Kwack et al. 2009	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Sprague-Dawley rats (male)	0, 500	Gavage, 28 days; DINP-2 (28553-12-0)	LOAEL: 500	Significant decrease in body weight and increase in relative liver weight accompanied by higher levels of glutamate oxaloacetate transaminase (GOT), alkaline phosphatase and triglycerides

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Ma et al. 2014	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Kunming mice (male)	0, 0.2, 2, 20, 200	Gavage, 14 days	NOAEL: 20 LOAEL: 200	Histopathological changes in the liver and kidney
Ma et al. 2015	(Environment Canada Health Canada, 2020; United States Environmental Protection Agency, 2022)	Male Kunming mice	0, 0.2, 2, 20 or 200 mg/kg-bw/day	Oral	LOAEL = 20 mg/kg bw/d	Histopathological alterations in pyramidal cells in the hippocampus, oxidative stress and inflammation in the brain; impaired cognitive ability was observed in MWM test and anxiety in Open field test at the 200 mg/kg bw/d
Moore 1998b	(Environment Canada and Health Canada, 2015)	B6C3F1 Mice	0, 1377 (male); 0, 1501 (female); (estimated from 0, 8000 ppm)	Diet, 78 weeks + 26 weeks regeneration; DINP-1 (68515-48-0)		Increased incidence of liver neoplasms in both sexes, significantly increased incidence of carcinoma in females only
Moore 1998b	(California EPA et al., 2013; Environment Canada and Health Canada, 2015; NTP, 2003; United States Environmental Protection Agency, 2022)	B6C3F1 Mice	0, 90, 276, 742,1560 (male); 0, 112, 336, 910, 1888 (female); (estimated from 0, 500, 1500, 4000, 8000 ppm).	Diet, 2 years; DINP-1 (68515-48-0)	NOAEL: 90	Non-neoplastic findings: increase in absolute liver weights accompanied by histopathological changes in the liver at the highest dose and decreased body weight gain in females from 336 mg/kg bw/d, increase incidence of liver masses and decreased absolute kidney weights in males from 276 mg/kg bw/d.
Peng 2015	(Environment Canada Health Canada, 2020)	Male Kunming mice	0, 1.5, 15, 150 mg/kg-bw/day	Oral	LOAEL = 150 mg/kg-bw/day	Reduced body weight gain, impaired cognitive ability in Morris water maze (MWM) test, histological alterations in pyramidal cells in the hippocampus

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Pugh et al. 2000	(Environment Canada and Health Canada, 2015; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	Macaca fascicularis Monkeys (male)	0, 500	Gavage, 14 days; DINP-1 (68515-48-0)	LOAEL: 500	Significantly increased neutrophil and decreased lymphocyte count
Qin et al. 2020		SPF BALB/c mice (male)	60 mg/kg, OVA+0,15; 3; 60 mg/kg DINP	Dermal exposure, 50 days; OVA + DINP	Not specified	Increasing dosage of DINP increased epidermal and dermal layer, as well as an increased expression of thymic stromal lymphopoetin in the skin. Increased IL-33 expression and decreased IFN-γ-expression were also observed at increasing dosage levels. IL-4, IL-5 and IL-13 expression were increased at the highest OVA+DINP dosage level (60mg/kg DINP).
Shellenberg et al. 1983	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Fisher 344 rats	0, 150, 500, 1500 (males); 0, 125, 420, 1300 (females)(estimated from 0, 0.2, 0.67, 2%).	Diet, 28 days, DINP-2 (28553-12-0)	LOEL: 125-150	Increased liver enzyme activity
Smith et al. 2000	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Fischer 344 rats (male)	0, 1000, 12 000	Diet, 2 or 4 weeks; DINP-1 (68515-48-0), DINP-A (71549-78-5)	NOEL: 1000 ppm LOEL: 12000 ppm	Increase in relative liver weight, increase in PBOX (peroxisomal beta-oxidation), inhibition of GJIC (gap junction intracellular communication) after 2 weeks (DINP-A) and after 4 weeks (DINP-1); increase in periportal DNA synthesis and centrilobular DNA synthesis

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Smith et al. 2000	(Environment Canada and Health Canada, 2015)	B6C3F1 Mice (male)	0, 500, 6000 ppm	Diet, 2 or 4 weeks; DINP-1 (68515-48-0)	NOEL: 500 ppm LOEL: 6000 ppm	Increase in liver weight, increase in PBOX activity and inhibition of GJIC
Unnamed 1969	(ECHA registration dossier, 2022)	New Zealand White (male/female)	0.5 or 2.5 mg/kg bw	24 hours per day, five days per week for six weeks	NOAEL: 500	Mild dermal irritation, but no systemic effects on kidney or liver
(Deng et al. 2019)		C57/BL6 mice (male)	0, 0.15; 1.5, 15	Gavage Six weeks		Relationship between DINP exposure and hypertension was investigated, each exposure with and without 1 mg/kg dexamethasone, DINP alone and in combination with dexamethasone could lead to hypertension by leading to increased ACE expression and to a dose-dependent increase in systolic, diastolic and mean blood pressure, which was 17 % higher in the highest dose group than in the control group (133 ± 2 mmHg).
(Chiu et al. 2020)		CD-1 mice (female)	0; 0.02; 0.2; 2; 20, 200	Gavage 10-14 days		Lowest dose environmentally relevant according to authors, tissue samples of colon collected during the dioestrus phase for morphological, biochemical and hormonal studies, significant colon damage in all exposure groups, with cell infiltration at lower doses and oedema at higher doses, decreased levels of oestradiol (significant at 0.2; 20 and 200 mg/kg) and tight junction expression (200 mg/kg), altered cytokine levels and altered expression of cell cycle regulators in the colon

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Gu et al. 2021)		ICR mice (male)	0, 0.05, 4.8	Gavage Five weeks	NOAEL: 0.05	Test for renal damage, increased levels of ROS, malondialdehyde and decreased GSH in the kidneys at HD, indicating oxidative damage, supported by sign. increased inflammatory cytokines TNF-α, but IL-6 was not increased
(Hwang et al. 2017)		C3H/HeN mice (female)	0, 2, 20, 200	I.p. injection six weeks		Effects on osteopenia investigated, bw, ALP, IP sign. but not dose dependently increased; activity of tartrate-resistant acid phosphatase (bone resorption marker) increased; activity of bone alkaline phosphatase decreased; microarchitecture of femur and tibia destroyed Tissue volume, bone volume, bone surface area, trabecular thickness, trabecular number decreased dose-dependently. Trabecular pattern factor, structural model index, trabecular separation increased; bone mineral density of femur and tibia lower in the treatment group
(C. Li et al. 2020)		Balb/c mice (male)	0, 2, 20, 200	Gavage 15 days	NOAEL: 2	Mice were sensitised to 5 mg OVA on day 1, 4, 7, 10 and 13 and challenged with a 1 % OVA aerosol for 30 min each from day 17-23. Lung tissue damage, airway hyperresponsiveness Immune molecule disruption was observed which may result in allergic asthma. According to the authors, adjuvant effect of DINP may be due to an imbalance in the

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						Th1/Th2 immune system, in which oxidative stress may also play a role. Effects were dose-dependent and significantly more pronounced above 20 mg DINP/kg + OVA than with OVA alone
(Liang and Yan 2020)		Balb/c mice (male)	0.02, 0.2, 2, 20, 200	Dermal 28 days		Levels of ROS, malondialdehyde and DNA-protein cross-links increased dose-dependently GSH levels decreased accordingly Effects significant above 20 mg/kg significant dose-dependent wrinkles and white vesicles in skin from 20 mg/kg on day 28
(Wu et al. 2015) **(Kang et al. 2016; 2017)**		Balb/c mice (male)	0, 1.4, 14, 140	Dermal 40 days then three times 0.5% fluorescein isothiocyanate (FITC) on days 41, 42 and 47		HD DINP + FITC resulted in increased infiltrating inflammatory cells in the skin compared to FITC alone. Skin swelling in all FITC-sensitised animals 24 hours after challenge Skin lesions previously induced by DINP exacerbated the topical sensitisation induced by FITC. Comparative control group with only DINP-exposed animals was missing – significance of the study is limited. Similar studies with comparable results were conducted by (Kang et al. 2016; 2017).

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Ma et al. 2014)		Kunming mice (male)	0, 0.2, 2, 20, 200	Gavage 14 days	NOAEL: 20	ROS accumulation in liver and kidney tissue (sign.at 200 mg/kg), GSH decreased accordingly (sign. from 20 mg/kg) No adverse effects if 50 mg/kg melatonin given in addition
(Chiu et al. 2021)		CD-1 mice (female)	0, 0.02, 0.2, 2, 20, 200	Gavage 14 days		Assessment of effects on colonic epithelial cells Increased protein levels of Ki67, MUC2, increased expression of a Paneth cell marker (Lyz1) and serum oestradiol levels, cytokines IL-1RA and CXCL12 also significantly altered in all exposure groups, more pronounced >20 mg/kg Exposure increases circulating oestradiol levels and gene expression in specialised epithelial cells according to authors.
(Kang et al. 2018)		Balb/c mice (male)	sensitised i.p. with 5 mg ovalbumin on days 1, 7 and 13 exposed via gavage to 20 mg/kg bw/d DINP from days 1-18	Gavage	LOAEL: 20	Exposure slightly worsened airway wall remodelling, promoted IgE and IgG1 production, induced appearance of airway hyperresponsiveness. Additional exposure to formaldehyde massively enhanced the observed effects.
(Duan et al. 2018)		Balb/c mice (male)	5 mg ovalbumin every 5 days 20 mg/kg bw/d from day 1-18	Gavage		DINP slightly worsened remodelling airway walls, additional exposure to formaldehyde massively enhanced the effects + change in the pyramidal cells of the prefrontal cortex, as well as a significant increase in ROS and a significant reduction in SOD. DINP exposure after sensitisation by ovalbumin resulted in sign. increase in IL-1β and IL-17 levels.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Ma et al. 2015)		Kunming mice (sex not given)	0.2, 2, 20, 200 + 50 mg/kg melatonin and 200 mg/kg/T DINP + 50 mg/kg/T melatonin	Gavage 14 days		Pathological differences in hippocampal cells with increasing DINP exposure (disorder of the cells to swollen cells or the absence of tip dendrites, loss of Nissl substance), increased levels of caspase-3 and GFAP in 20 and 200 mg/kg group, increased expression of TNF-α and IL-1β, increased completion time of Morris water maze test at 200 mg/kg
(Peng, 2015)		Kunming mice (male)	0, 1.5, 15, 150 or 105 mg/kg DINP + 50 mg/kg/T vitamin E for 9 days	Gavage 9 days		In Morris water maze tests, over the training period of 7 days, all mice completed the tests faster, but the speed with which the mice learned was slower with increasing exposure, after a pause phase, mice with increasing exposure took longer to complete the test, Pyramidal cells in hippocampus showed increased disorder and deformation of the cells with increasing exposure, TNF-α and IL-1 more pronounced in HD
		NC/Nga mice (male)	6.6, 131.3, or 2625 µg/animal	S.c. injection 2-3 times a week of mite-allergen (Dermatophagoides pteronyssinus) to induce ADSLs (8 times in total) + once a week for four weeks the mice were orally exposed to DINP		Clinical scores of the Dp + DINP (6.6 and 131.3 µg/animal) exposure group began to increase from day 16. No significant interaction effect between dose and day was present. Eosinophil counts of the Dp + DINP 2625 µg/animal) group were similar to ctrl.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Unnamed 1971	(ECHA registration dossier, 2022)	Beagle dog (male/female)	0.125, 0.5, 2.0 %	Diet 13 weeks	LOAEL 37 (female)	Increased AST in females and increased liver weight, lowest dose tested 0.125 % in the diet (37 mg/kg/day)
Unnamed 2000	(ECHA registration dossier, 2022)	B6C3F1 Mice (male/female)	1500, 4000, 10000, 20000 ppm	Diet 13 weeks	NOAEL: < 341 (male), 389 (female) LOAEL: 341 (male), 1041 (female)	Significant treatment related absolute and relative organ weight changes were noted in the uterus (decrease), spleen, (decrease), liver/gallbladder (increase), kidney (decrease for the males but increase for the females), and testes/epididymites (decrease).

Table VI.40: Overview of studies on skin sensitization

Reference	Cited in	Findings
Butala et al. 2004	(NICNAS, 2012)	- Dermal administration of undiluted DINP to B6C3F1 mice - No significant elevations in total serum IgE, IL-4 or IL-13
CPSC 2010	(United States Environmental Protection Agency, 2022)	- DINP is not a dermal sensitizer
Exxon Biomedical Sciences 1992, ECB 2003	(NICNAS, 2012)	- Buehler test in 40 animals (20 treated + 20 ctrl) under occlusive conditions, challenge application with 5% DINP in peanut oil - Some evidence of sensitisation + score 2 erythema at day 37 in 3/20 animals (score 1 in 4 ctrl animals)
Huntingdon Research Centre 1998, ECB 2003	(NICNAS, 2012)	- Buehler test in 40 animals (20 treated + 20 ctrl) under occlusive conditions, challenge application of undiluted DINP - No signs of skin sensitisation
Koike et al. 2010 ND	(NICNAS, 2012)	- DINP was injected intraperitoneally at 0, 0.15, 1.5, 15 or 150 mg/kg/d in mice (strain not given) - At 15 mg/kg/d aggravation of atopic dermatitis (AD)-like skin lesions which were consistent with eosinophilic inflammation, mast cell degranulation and thymic stromal lymphopoietin (TSLP) expression in the inflamed ear
Larsen et al. 2002, ECHA 2010	(NICNAS, 2012)	- S.c. injection in BALB/cJ mice with 0, 2, 20, 200 or 2000 µg/ml in the neck region with ovalbumin, then 1-2 ovalbumin injections alone - Significant adjuvant effect on IgE and IgG after first booster at 200 µg/ml, dose dependent increased IgG antibody level at 200 and 2000 µg/ml after second booster, no increase in IgE

Table VI.41: Overview of genotoxicity studies

Reference	Cited in	Findings
Barber et al. 2000	(NTP, 2003; United States Environmental Protection Agency, 2022)	• Negative results for forward mutations at the TK locus in cultured L5178Y cells in assays conducted with and without activation by S9 rat liver fraction. The tested concentrations ranged from 1.5 to 8.0 µL/mL for assays in the absence of metabolic activation and from 0.05 to 0.6 µL/mL for assays in the presence of S9 fraction • DINP gave negative results when evaluated *in vitro* in Balb/c-3T3 A31 mouse cells at concentrations of 0.125 to 3.750 µL/mL in the absence of metabolic activation.
BASF 1986; BASF 1995	(United States Environmental Protection Agency, 2022)	• Negative results in tester strains TA98, TA100, TA1535, TA1537 in the presence and absence of DINP at concentrations ranging from 20 to 5000 µg/plate
EG&G Mason Research Institute 1982	(United States Environmental Protection Agency, 2022)	• Negative results for mutagenic activity in S. typhimurium tester strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence and absence of Aroclor-induced rat liver S9 fraction in the plate incorporation assay at DINP concentrations ranging from 0.1 to 10 µL/plate • *In vitro* testing using mouse L5178Y lymphoma cells. DINP did not induce forward mutations at the thymidine kinase (TK+/−) locus of L5178Y cells when tested at concentrations ranging from 7.5 to 100 µL/mL in the presence and absence of metabolic action by Aroclor-induced rat liver S9 fraction
Exxon Biomedical Sciences 1996, reviewed in EC JRC 2003;	(BAuA, 2001; California EPA et al., 2013; NICNAS, 2012)	• DINP was negative for chromosomal aberrations in CHO cells in *vitro* in the presence or absence of an exogenous metabolic system (Aroclor-induced SD rat liver S9) after exposure to 5, 10, 20, 40, 80, 160 µg/mL
Exxon Biomedical Sciences 1996b	(NICNAS, 2012)	• Ames test in S. typhimurium TA 98, 100, 1535, 1537, 1538 at 0.5 to 5,000 µg/plate ± S9 negative
Fushiwaki et al. 2003	(United States Environmental Protection Agency, 2022)	• Negative results for tumour-promoting potential in Balb/c-3T3 mouse cells co-cultured with transformed cloned cells (strain 4-1-1) and treated with DINP at concentrations of up to 5.0 µg/mL
Hazleton 1986b, reviewed in EC JRC 2003; **Barber et al. 2000**	(BAuA, 2001; California EPA et al., 2013; NICNAS, 2012)	• Mouse lymphoma assay negative at 1,500 to 8,000 nL/mL without metabolic activation and 500 to 6,000 nL/mL with metabolic activation • DINP did not induce forward mutations in L5178Y TK +/- mouse lymphoma cells in studies performed with and without activation by S9
Litton Bionetics 1981	(United States Environmental Protection Agency, 2022)	• DINP gave negative results when evaluated *in vitro* in Balb/c-3T3 A31 mouse cells at concentrations of 2.5 to 254.5 µg/mL in the absence of metabolic activation.

Reference	Cited in	Findings
Litton Bionetics 1981	(BAuA, 2001; NICNAS, 2012)	• Negative in a mammalian unscheduled DNA synthesis test in rat hepatocytes from 0.625 to 10 µg/l
Litton Bionetics 1982	(United States Environmental Protection Agency, 2022)	• DINP gave negative results when evaluated *in vitro* in Balb/c-3T3 A31 mouse cells at concentrations of 0.2 to 3,260 µg/mL in the absence of metabolic activation.
Litton Bionetics 1985	(BAuA, 2001; United States Environmental Protection Agency, 2022)	• DINP gave negative results when evaluated in vitro in Balb/c-3T3 A31 mouse cells at concentrations of 125 to 3,750 nL/mL, in the absence of metabolic activation.
McKee et al. 2000	(United States Environmental Protection Agency, 2022)	• Negative results for mutagenicity in S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 when tested at DINP concentrations of 0.5 to 5000 µg/plate • DINP was tested for clastogenic activity in cultured Chinese hamster ovary (CHO) cells at concentrations of 40, 80, and 160 µg/mL (in acetone) in the presence or absence of an exogenous metabolic system from Aroclor-induced Sprague Dawley rat livers: DINP did not induce chromosomal aberrations in CHO cells • Negative results were obtained for induction of micronuclei in male CD-1 rats (5/dose) given DINP by oral gavage at concentrations of 500, 1,000, or 2000 mg/kg for 2 days
McKee et al. 2000; reviewed in US EPA 2005a	(California EPA et al., 2013)	• DINP was negative in the *in vivo* micronucleus test in male CD-1 mice exposed to 500, 1000 and 2000 mg/kg for two days and in F344 rats receiving 0.5, 1.7 and 5 mg/kg/day DINP in maize oil for five days.
Microbiological Associates 1981	(United States Environmental Protection Agency, 2022)	• DINP gave negative results when evaluated *in vitro* in Balb/c-3T3 A31 mouse cells. Slight increases in the number of transformed foci were observed in Balb/c-3T3 A31 cells treated with 0.1 to 1 µL/mL in the absence of metabolic activation. However, the responses were not dose-related or statistically significant.
Microbiological Associates 1981a	(NICNAS, 2012)	• Cytogenetic assay in bone marrow cells of F344 rats negative with 0.5, 1.7 and 5 mg/kg bw/d during 5 days via oral route
Microbiological Associates 1982	(United States Environmental Protection Agency, 2022)	• Frequency of chromosomal aberrations in femoral bone marrow cells of male Fischer-344 rats (5/dose) given DINP by oral gavage once daily for 5 days consecutively • Administration of 0.5. 1.7, or 5.0 mg/kg/day in corn oil did not induce statistically significant clastogenic effects
Microbiological Associates 1982	(United States Environmental Protection Agency, 2022)	• Positive results were observed in one *in vitro* transformation assay of Balb/c 3T3 cells conducted at concentrations of 0.03 to 1 µL/mL in the absence of metabolic activation.

Reference	Cited in	Findings
Microbiological Associates 1982	(United States Environmental Protection Agency, 2022)	• DINP gave negative results when evaluated *in vitro* in Balb/c-3T3 A31 mouse cells. Slight increases in the number of transformed foci were observed in Balb/c-3T3 A31 cells treated with 0.03 to 1 µL/mL without metabolic activation. However, the responses were not dose-related or statistically significant.
Microbiological Associates 1982	(United States Environmental Protection Agency, 2022)	• DINP gave negative results when evaluated *in vitro* in Balb/c-3T3 A31 mouse cells. Slight increases in the number of transformed foci were observed in Balb/c-3T3 A31 cells treated with 0.1 to 1 µL/mL in the presence. However, the responses were not dose-related or statistically significant.
Reviewed by EC JRC 2003	(California EPA et al., 2013)	• DINP in Salmonella typhimurium (test strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538) with and without metabolic activation. • No positive response was observed in the presence or absence of metabolic activation.
Zeiger et al. 1985	(BAuA, 2001; United States Environmental Protection Agency, 2022)	• Negative results in tester strains TA98, TA100, TA1535, and TA1537 at DINP concentrations of 0 to 10,000 µg/plate in the presence and absence of rat or hamster liver S9 fraction

Table VI.42: Overview of carcinogenicity studies

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	Findings
Bio/dynamics 1986	Palmer(Environment Canada and Health Canada 2015; United States Environmental Protection Agency 2022; California EPA et al. 2013; NICNAS 2012; BAuA 2001)	Sprague-Dawley rats	0, 27, 271, 553 (male); 0, 33, 331, 672 (female) (estimated from 0, 500, 5000, 10 000 ppm).	Diet, 2 years; DINP-A (71549-78-5)	• Increase in the incidence of hepatocellular carcinoma in females treated with the two highest doses. • Increased incidence of neoplastic hepatic nodules at all doses (not significant). • Significant increase in testicular interstitial cell hyperplasia in males at the highest dose. Non-significant increase in the incidence of testicular interstitial cell carcinoma (increase was outside the range of the historical control). • Slight increase in pancreatic islet cell carcinoma and parathyroid hyperplasia in males treated with the highest dose. • Slight increase in endometrial hyperplasia and adenocarcinoma in high-dose females. • LOAEL (non-neoplastic): 27 mg/kg bw/d (histological changes in liver) (male) • LOAEL (neoplastic): 271-333 mg/kg bw/d (hepatocellular carcinomas)
Butala et al. 1996	(BAuA, 2001; Caldwell, 1999)	F-344 rats	0, 500, 1,500, 6,000, 12,000 ppm	Diet, 2 years, chronic	• Statistically significant increase in mononuclear cell leukaemia (MNCL) in dose groups treated with 0.6 and 1.2% in males and females, although within historical control range. • NOEL of 0.15% (90 mg/kg/day) was considered for all endpoints.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	Findings
(Lington et al., 1997)	(BAuA, 2001; California EPA et al., 2013; Environment Canada and Health Canada, 2015; Environment Canada Health Canada, 2020; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	F-344 rats(10/sex/ dose) Conducted according to the GLP standards of the US EPA	0, 15, 152, 307 (males); 0, 18, 184, 375 (females) (estimated from 0, 0.03, 0.3, 0.6%).	Diet, 2 years; DINP-1 (68515-48-0)	• Ten rats/sex/dose were killed at 6, 12 and 18 months and the remaining rats at 24 months • No treatment-related effects at 6 and 12 months, minimal to slight centrilobular enlargement of hepatocytes in the centre of the liver after 18 months • No increased mortality in any dose group • Increased incidence of hepatocellular carcinoma and a positive dose-response trend for hepatocellular carcinoma (p=0.015) in HD males • Increased incidence of MNCL at the two highest doses tested in both sexes + renal neoplasms (transitional cell carcinomas and tubular cell carcinomas. • Dose-dependent significant increase in the incidence of MNCL observed in female rats • LOAEL (non-neoplastic): 152 - 184 mg/kg bw/d (increase in absolute and relative liver and kidney weights and increase in histopathological changes in both organs at the two highest doses).
(Caldwell, 1999)					• Re-evaluation of (Lington et al., 1997) and (Butala et al. 1996) to assess the significance of the occurrence of MNCL as a response to alkyl phthalate treatment in humans • According to author, MNCL commonly occurs in F-344 rats + evidence for a threshold at a level not biologically relevant, which must be exceeded before carcinogenic effects occur • Author concluded that based on weight of evidence, the data is not sufficient to assume a carcinogenic hazard to humans and that classification of DINP as a carcinogen is not scientifically supported

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	Findings
					• The kidney tumours are likely explained by an alpha 2µ-globulin-dependent mechanism since treatment with DINP had resulted in a dose-dependent accumulation of alpha 2µ-globulin in male rat kidneys and are therefore not considered relevant for humans
Moore 1998a	(California EPA et al., 2013; Environment Canada and Health Canada, 2015; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	Fisher 344 rats	0, 29, 88, 359, 733 (male); 0, 36, 109, 442, 885 (female); (estimated from 0, 500, 1500, 6000, and 12 000 ppm)	Diet, 2 years; DINP-1 (68515-48-0)	• Dose-dependent increase in incidence of mononuclear cell leukaemia in both sexes above 6000 ppm. • Significant increase in hepatocellular carcinomas in males at the highest dose tested, but not in females. Significant increase in the incidence of carcinomas or adenomas in both sexes at the highest dose. • LOAEL (non-neoplastic): 358 - 442 mg/kg bw/d (increase in absolute and relative liver and kidney weights, increase in serum ALT and AST and histopathological findings in both organs) (both sexes).
Moore 1998a Regeneration Study	(Environment Canada and Health Canada, 2015; United States Environmental Protection Agency, 2022)	Fisher 344 rats	0, 637.3 (males); 0, 773.6 (females) (estimated from 0, 12 000 ppm)	Diet, 78 weeks + 26 weeks regeneration	• Significant increase in MNCL in both sexes and significant increase in renal tubular carcinoma in males.
Moore 1998b	(California EPA et al., 2013; Environment Canada and Health Canada, 2015; NTP, 2003; United States Environmental Protection Agency, 2022)	B6C3F1 Mice	0, 90, 276, 742,1560 (male); 0, 112, 336, 910, 1888 (female); (estimated from 0, 500, 1500, 4000, 8000 ppm).	Diet, 2 years; DINP-1 (68515-48-0)	• Significant increase in the incidence of hepatocellular carcinomas at the two highest doses in females and at the highest dose in males. Significant increase in the incidence of total liver neoplasms (carcinomas and adenomas) in females from 336 mg/kg bw/d and in males at the two highest doses. • LOAEL (non-neoplastic): 276 - 336 mg/kg bw/d (increase in absolute liver weights accompanied by histopathological changes in the liver at the highest dose and decreased body weight gain) (female); (increase in

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	Findings
					incidence of liver masses and decreased absolute kidney weights) (male).
Moore 1998b Regernation 's study	(Environment Canada and Health Canada, 2015)	B6C3F1 Mice	0, 1377 (male); 0, 1501 (female); (estimated from 0, 8000 ppm)	Diet, 78 weeks + 26 weeks regeneration; DINP-1 (68515-48-0)	• Increased incidence of liver neoplasms in both sexes. Significantly increased incidence of carcinoma in females only.
Pathology Working Group Review of Data for Spongiosis Hepatis and MNCL.EPL	(United States Environmental Protection Agency, 2022)	selected lesions of the liver and spleen observed in Fischer-344 rats in the 2-year bioassays reported by Lington et al. and Moore	See Lington et al and Moore		• Confirm the original findings of the study pathologist(s); the NOAEL for DINP is 1,500 ppm (88 mg/kg/day) in male rats, based on the increased incidence of hepatic spongiosis in males at concentrations of 3,000 ppm (152 mg/kg/day) and greater. The PWG identified a NOAEL of 3,000 ppm (184 mg/kg/day) in females, based on increased incidence of MNCL at concentrations of 6,000 ppm (375 mg/kg/day) or higher

Table VI.43: Overview of studies regarding effects on fertility

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Amjad et al., 2021)		ICR mouse	1, 10, 100 and 500 µg/ml	*In vitro*, 90 min		Effect of DINP on spermatozoa *in vitro* Mixture of DINP (15%), DEP, DBP, DiBP, DEHP and DiDP Sign. limitations of motility by reducing the ability to generate ATP Only mixture tested
(Barakat et al., 2019)		CD-1 mice	0.02, 0.2, 200 or 500	Oral GD 10.5 until parturition		Phthalate mixture of DINP, DEHP, DEP, DBP, DiBP and BBzP found in the urine of pregnant women No clear adverse effects Only mixture tested
(Boberg et al., 2016, 2011) **(M. Chen et al., 2017)**		Wistar rats	0, 300, 600, 750, 900	Gavage GD 7 – PND 17		F1 males examined at PND 90 Histopathological changes in the testes (multinucleated germ cells, increased number of gonocytes, reduced sperm motility, increased sperm count, in a dose-dependent manner No effects on gestational length, post implantation losses, litter size, sex ratios or perinatal losses in females Maternal bw unaffected anogenital distance of males at PND 1 decreased dose dependently Statistical significance of the histopathological changes, the anogenital distance and the collected sperm parameters was questioned in a re-evaluation by Chen et al. (2017)

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Chiang and Flaws, 2019)		CD-1 mice	0, 0.02, 0.2, 20, 200	Oral 10 days		Females were mated with untreated males immediately after exposure and again after three and nine months Sign. disturbed oestrus cycles at 0.02, 0.2 and 200 mg/kg, females became pregnant significantly less often at the lowest dose Nine months after exposure, at 0.2 mg/kg and above, the sex ratio was shifted towards males No dose dependency in the effects, variability within animals was very pronounced
(Chiang et al., 2020a)		CD-1 mice	0, 0.02, 0.1, 20, 200	Oral 10 days		12 and 15 months after exposure, the mice were mated with untreated males similar results were obtained, but with the same limitations as before (Chiang and Flaws 2019) Exposure duration is titled once as subchronic and once as short-term exposure, but in both cases includes a duration of 10 days
(Chiang et al., 2020b)		CD-1 mice	0, 0.02, 0.1, 20, 200	Oral 10 days		Repeated experiment like before or re-evaluation hormones and folliculogenesis in the ovaries were determined three, six and nine months after exposure change in the distribution of follicular populations in the ovaries and of the sex steroid hormones oestradiol, testosterone, progesterone, FSH and inhibin B at different time points, including the last time point according to authors. No dose dependencies, only isolated values are statistically significantly altered from control and with considerable variability.

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(J. Chen et al., 2022)		Female Kunming mice	0, 2, 20, 200	Oral 14 days		Effects on the female reproductive organs were investigated. According to authors, DINP led to a sign. impairment in arrangement of granulosa cells in ovarian follicles apoptosis, autophagy and oxidative stress induced in ovarian tissue in a dose-dependent manner. Serum oestradiol concentration decreased sign. in the MD and HD groups, suggesting, that DINP may affect the function of ovarian granulosa cells.
(Earl Gray, 2023)		Female Crl:CD(SD) rats	0, 1000, 1500 + 750 taken from a previous study	Gavage GD 14 – PND 3		No maternal toxicity Mild but significant malformations of the testes and epididymides in the offspring Hypospermatogenesis/atrophy of the testicular urinary bladder in a dose-dependent manner According to the authors, DINP had demasculinising effects on the androgen-dependent organs
(Li et al., 2015)		Female SD rats	0, 10, 100, 500, 1000	Gavage GD 12-21		Testosterone production of the testes, number, and distribution of foetal Leydig cells, gene and protein expression of the testes were investigated in male offspring at GD 21.5. Dose-dependent increase in aggregation and significant enlargement of foetal Leydig cells from 10 mg/kg and multinucleated gonocytes from 100 mg/kg, gene expression and protein levels of insulin-like 3 and 3b-hydroxysteroid dehydrogenase were inhibited from 10 mg/kg. Testicular testosterone production reduced at 1000 mg/kg; testicular volume not affected

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Neier et al., 2020)		Yellow agouti mice	0, 75	2 weeks before mating until PND 21		Metabolic influences of perinatal exposure to DINP investigated, one male and one female F1 mouse per litter kept without further exposure until 10 months, impaired glucose tolerance and altered relationship between body fat percentage and plasma adipokine levels in females, not in males; reanalysis of the data revealed that perinatal exposure resulted in increased body and liver weights in male and female offspring at PND 21 (sign. in females), according to authors, it is associated with short- and long-term activation of PPAR target genes, resulting in increased fatty acid production in early postnatal life and increased fatty acid oxidation in adulthood.
(Rajkumar et al., 2022)		Different gonadal somatic and germ cell lines, Sertoli cell line (TM4), male spermatogonia cell line (C18-4), two steroidogenic cell lines (MA-10 Leydig and KGN granulosa)	0.001, 0.01, 0.1, 1, 5, 10, 20, 50, 100 µM	*In vitro* 48 hours		Cytotoxicity, cellular oxidative stress, mitochondria and lysosomes in the cells were investigated. Calcein intensity in C18-4 cells was increased in concentration-dependent manner, sign. above 50 µM, proportion of active mitochondria sign. increased in concentration-dependent manner above 20µM, significantly increased number of lipid droplets and oxidative stress + proportion of active mitochondria in KGN cells at 100 µM
Unnamed 2015	(ECHA registration dossier, 2022)	Wistar rat (albino)	0, 276, 1380	Gavage	1380	No toxicologically relevant estrogenic effect on uterus, and ovary weight, and did not induce precocious vaginal opening

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Unnamed 2018	(ECHA registration dossier, 2022)	Wistar rat (female)	0, 380	Gavage	-	Findings indicate that in utero and lactational exposure to DINP negatively impacts the maturation of the small intestine in offspring, in the presence of severe maternal malnutrition observed during gestation and lactation.
Waterman et al., 2000	(BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	SD rats (30/sex/group) One-gen study	0, 0.5, 1.0, 1.5 % w/w (0, 301–923, 622–1731, 966–2246 mg/kg bw/d)	Diet, 10 weeks prior to mating till weaning, females exposed throughout gestation and lactation until PND 21	NOAEL (fertility): 1.5 % (~1,000 mg/kg bw/d)	No significant effects on indices of mating or fertility (e.g., litter size)
Waterman et al., 2000	(BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	SD rats (30/sex/group) 2-gen study	0.0, 0.2, 0.4, or 0.8 % (0, 114–395, 235–758, 467–1541 mg/kg bw/d)	Diet, 10 weeks before mating and for an additional 7 weeks, through mating, gestation, and lactation	NOAEL (fertility): 0.8 % (467 - 1541 mg/kg bw/d)	No significant effects on indices of mating or fertility

Table VI.44: Overview of studies regarding developmental toxicity

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(van den Driesche et al., 2020)		Wistar rats	0, 125, 750	Gavage GD 15.5 – 18.5	NOAEL: 750	In utero exposure to study effects during window of masculinisation, no maternal tox, litter size and sex ratio not affected DINP did not cause cryptorchidism or hypospadias, had no effects on anogenital distance or Leydig cell aggregates. No reduction in intratesticular testosterone, no effects on sperm motility, sperm count, testosterone or LH levels in adults
(Yang et al., 2021)		Kunming mice	0, 4.8	Subcutaneous PND 0-21	LOAEL: 4.8	Study on effects on liver lipidomic changes in the liver, elevated di- and triglycerides and cholesterol esters indicating hyperlipidaemia, according to the authors
(Huang et al., 2019)		Kunming mice	0, 0.048, 4.8	Subcutaneous PND 0-21	NOAEL: 4.8	10 males and females were studied at PND 22 Significant sex-specific differences in the fatty acid compositions were found at 4.8 mg/kg, no further conclusions
(L. Chen et al., 2015)		Wistar rats	0, 5, 50, 500	Gavage PND 7-21		Offspring sensitised to 1 mg ovalbumin s.c. at PND 22, 23 and 37 Challenged to 1% OVA at PND 44 and 45 for 30 min each At 50 but not 500 mg/kg, the airway response to OVA was significantly increased, mice showed dramatically increased pulmonary resistance
(Clewell, Thomas, et al., 2013)		Sprague-Dawley rats	0, 50, 250, 750 (0, 760, 3800, 11.400 ppm)	Diet GD12-PND14	NOEL based only on statistically significant effects present in weaned animals: 750	Maternal bw and food consumption decreased in HD on GD20, PND2 and 14 Sign. decreased bw in HD male pups Testis and epididymis weights not affected increased incidence and severity of Leydig cell aggregation and decreased anogenital distance was observed in the testes of HD pups

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						Multinucleated germ cells were significantly increased in MD and HD animals Biological significance of these temporary effects is unclear
(Clewell, Sochaski, et al., 2013)		Sprague-Dawley rats	0, 50, 250, 500	Gavage GD 12-19	NOAEL: 50 ED_{50} of 250, IC_{50} (on the maternal plasma monoester) of 51 µM, and an IC_{50} (on the foetal plasma monoester) of 42 µM were derived	Disposition of diisononyl phthalate and its effects on sexual development was investigated DINP metabolites MINP, MciOP, MhiNO, MoiNP, MiNP-G found in all measured tissues Abs. and rel. maternal Liver weight sign. increased in MD and HD Foetal weight unaffected No spontaneous abortion, increased incidence of fatal resorption or changes in anogenital distances in any dose group Sign. increased multinucleated germ cells in HD Sign. reduction of testosterone in male foetuses in MD and HD groups at 2h post-dosing
Adamsson et al., 2009	(United States Environmental Protection Agency, 2022), (NICNAS, 2012)	SD rats (7-8/group) Developmental tox study	0, 250, 750	Gavage GD 3-17	NOAEL: 250	Exposure to DINP shows demasculinization with female-like nipples, small and atrophic testis and epididymal agenesis increased foetal testicular gene expression on GD 19 at 750 mg/kg bw/d
Boberg et al., 2011	(California EPA et al., 2013; NICNAS, 2012; United States Environmental Protection Agency, 2022)	Wistar rats (16/group)	300, 600, 750, 900	Gavage GD 7 – PND 17	NOAEL: 300	No maternal toxicity was observed 600 mg/kg bw/d: increased nipple retention, increased testicular pathology (MNG), decreased testicular testosterone content & production (not statistically significant), decreased sperm motility 750 mg/kg bw/d: decreased pup weight PND 13 (f), decreased testicular pathology (enlarged seminiferous tubule) 900 mg/kg bw/d: decreased pup weight PND 13 (m), decreased AGD & AGI, decreased testicular testosterone

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						PND 90 (37%, not statistically significant), increased sperm counts, masculinised learning behaviour (f)
Borch et al., 2004	(California EPA et al., 2013; NICNAS, 2012; United States Environmental Protection Agency, 2022)	Wistar rats (8/group) hormonal effects of DINP in male rat foetuses	0, 750	Gavage GD 7-21	LOAEL: 750	Testicular testosterone content and testicular testosterone production (*ex vivo*) were significantly reduced (26%) on GD 21, while plasma luteinising hormone (LH) was significantly increased compared to controls.
Clewell 2011	(NICNAS, 2012; United States Environmental Protection Agency, 2022)	Sprague-Dawley rats (20/group, 24 controls)	0, 760, 3,800, or 11,400 ppm (target doses of 0, 50, 250, or 750 mg/kg bw/d, respectively)	Diet GD 12 – PND 14	NOAEL (maternal): 3,800 ppm (~250 mg/kg bw/d) NOAEL (development): 760 ppm (~50 mg/kg bw/d)	Reduced body weight, body weight gain, and food consumption during gestation and lactation at 750 mg/kg bw/d, decreased pup weight >3,800 ppm, increased MNGs at 11,400 ppm, decreased AGD and increased LC aggregation
Clewell et al. 2011	(NICNAS, 2012; United States Environmental Protection Agency, 2022)	SD rats (8/group)	0, 50, 250, 750	Gavage GD 12-19	NOAEL (maternal + development): 50	Maternal effects comprised of increased liver weights at 250 mg/kg bw/d. Increased testicular pathology (MNG) at 250 mg/kg bw/d, decreased foetal testicular testosterone on GD 19, increased on GD 20 Increased no. of gonocytes and size of Leydig cell aggregates at 750 mg/kg bw/d
Clewell et al. 2013	(Environment Canada and Health Canada, 2015; Environment Canada Health Canada, 2020)	Rats	Up to 750	GD 12-19		In urine, MCIOP was the major metabolite, while MINP and its glucuronidated form (MINP-Gluc) were almost negligible. In contrast, all metabolites were present in maternal plasma (MINP in similar concentrations to MCIOP). It was concluded that urinary excretion of MINP and MINP-Gluc is limited and that these urinary metabolites are poor predictors of plasma and tissue disposition of DINP.

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						MINP and its oxidative metabolites as well as its glucuronidated form were all present in foetal plasma, testis and amniotic fluid. MINP-Gluc was the only metabolite present in higher concentrations in the foetal plasma than in the maternal plasma, indicating a possible placental transfer of this form or, more likely, that conjugation could take place in the foetus.
(Furr et al., 2014)	(United States Environmental Protection Agency, 2022)	Harlan or Charles River SD rats short-term in vivo screening study	950	Gavage GD 14-18	LOAEL: 950	No effect on foetal viability or dam weight gain, statistically significant 50 % reduction in ex vivo foetal testis testosterone production
Gray et al., 2000	(California EPA et al., 2013; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	Rats (6-8/group)	0, 750	Gavage GD 14 – PND 3	LOAEL (maternal + development): 750	Decreased maternal bw gain to GD 21 significant increased retained areolas/nipples observed in 22 % of the male offspring. In addition, some malformations of the reproductive system occurred in 7.7 % of the male offspring, including small and atrophic testes, fluid-filled testes, unilateral epididymal agenesis with hypospermatogenesis and fluid-filled testes without spermatids in the scrotum
Hannas et al., 2011	(California EPA et al., 2013; NICNAS, 2012; United States Environmental Protection Agency, 2022)	Harlan SD rats	0, 500, 750, 1000, 1500 DINP or DINP-2	Gavage GD 14-18	LOAEL: 500	Decreased foetal testicular testosterone production (GD 14–18) at 500 mg/kg Decreased foetal testicular gene expression (StAR & Cyp11a) (GD 14-18) at 1000 mg/kg
Hannas et al., 2012	(California EPA et al., 2013; NICNAS, 2012)	Harlan SD rats	0, 500, 750, 1000, 1500 DINP or DINP-2	Gavage GD 14-18	LOAEL: 500	Decreased Insl3 & other foetal testicular gene expression for androgen synthesis and cholesterol transport (GD 14–18) at 500 mg/kg bw/d

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Hazleton 1981	(NICNAS, 2012)	SD rats (25/group)	500, 1000	Gavage GD 6-15	NOAEL (maternal + development): 500	No developmental effects observed, no maternal toxicity
Hellwig et al., 1997	(BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	Wistar rats (10/group)	0, 40, 200, 1,000	Gavage GD 6-15	NOAEL (maternal + development): 200	Dams were sacrificed on GD 20. Maternal toxicity at 1,000 mg/kg bw/d and sign. reduced food consumption during several days, reduced mean bw gain from GD 6-15, sign. reduced mean bw at study termination and significantly increased relative liver weight. No treatment-related effects occurred in the dams receiving doses of 200 or 400 mg/kg/day. Skeletal variations (rudimentary cervical & accessory 14th ribs) with DINP1 & DINP2 at 1000 mg/kg bw/d
(Li et al., 2015)	(Environment Canada Health Canada, 2020; United States Environmental Protection Agency, 2022)	SD rats (6/group) study investigated effects of DINP on foetal Leydig cell function and testis development.	10, 100, 500, 1000	Gavage GD 12-21	LOAEL: 10	Dose-dependent increase in aggregation and significant enlargement of foetal Leydig cells from 10 mg/kg and multinucleated gonocytes from 100 mg/kg Gene expression and protein levels of insulin-like 3 and 3b-hydroxysteroid dehydrogenase were inhibited from 10 mg/kg. Testicular testosterone production was reduced at 1000 mg/kg, testicular volume was not affected.
Lee and Koo 2007	(California EPA et al., 2013; Environment Canada Health Canada, 2020; NICNAS, 2012; United States Environmental Protection Agency, 2022)	SD rats (6/group) Testicular toxicity via an antiandrogenic mechanism was investigated in Hershberger assay	0, 20, 100, 500	Gavage 10 days (period unknown)	LOAEL: 20	Groups of six-week-old SD rats were given 0.4 mg/kg/day testosterone propionate after castration. After one week of recovery, the rats were orally administered DINP. No significant effects were observed on absolute body, liver, kidney or adrenal weights compared to testosterone-treated rats. The absolute weights of the seminal vesicles and levator ani/bulbocavernosus were significantly reduced at all DINP doses compared to testosterone controls.

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Lee et al., 2006	(California EPA et al., 2013; NICNAS, 2012; United States Environmental Protection Agency, 2022)	Wistar rats	0, 40, 400, 4000, 20,000 ppm (0, 2, 20, 200, 1,000 mg/kg bw/d)	Diet GD 15 – PND 21	LOAEL: 2	No maternal toxicity 2 mg/kg bw/d: decreased pup weight, decreased anogenital distance and anogenital index, increased hypothalamic grn and p130 mRNA levels at PND 7 in female and male animals, respectively. The lordosis quotient was decreased in female animals perinatally exposed to DINP and DBP, and DINP significantly decreased anogenital distance (AGD) in male animals at all doses used.
Masutomi et al., 2003	(California EPA et al., 2013; United States Environmental Protection Agency, 2022)	SD rats	400, 4,000, 20,000 ppm	Diet GD 15 – PND 10	NOAEL: 4,000 ppm	Degeneration of meiotic spermatocytes and Sertoli cells in the testes and a decrease in the corpus luteum in the ovary at 20,000 ppm at week 11
Masutomi et al., 2004	(California EPA et al., 2013; NICNAS, 2012)	Wistar rats expression of pituitary hormones was studied	0, 400, 4000, 20000 ppm (0, 31–66, 307–657, 1,165–2,657 mg/kg bw/d)	Diet GD15 – PND10	NOAEL (developmental): 31-56	Maternal food consumption and bw gain was decreased at top dose group Developmental: 307–657: decreased pup weight (m) PND 27; 1,165–2,657: decreased brain & pup weights (m-f), decreased testis, absolute ovarian & uterus weights PND 27, increased testicular pathology (degeneration of meiotic spermatocytes & Sertoli cells), decreased corpora lutea in the ovary PND 77
Ostby et al., 2001	(California EPA et al., 2013; NICNAS, 2012; NTP, 2003)	SD rats	0, 1000, 1500	Gavage GD14 – PND3	LOAEL: 1000	No maternal toxicity reported, increased nipple retention on day 13 of life with 55% at 1000 mg/kg and 75% at 1500 mg/kg compared to 14% in controls, decreased anogenital distance at 1500 mg/kg bw/d

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Waterman et al., 1999	(BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	SD rats (25/group)	0, 100, 500, 1,000	Gavage GD 6-15	NOAEL (maternal + developmental): 500	Decreased food consumption in dams at 1000 mg/kg bw/d, skeletal (rudimentary lumbar ribs) & visceral (dilated renal pelvis) variations at 1000 mg/kg bw/d
Waterman et al., 2000	(BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	SD rats (30/sed/group) One-gen study	0, 0.5, 1.0, 1.5 % w/w (0, 301–923, 622–1731, 966–2246 mg/kg bw/d)	Diet, 10 weeks prior to mating till weaning, females exposed throughout gestation and lactation until PND 21	LOAEL (development): 0.5 % (301–923 mg/kg bw/d)	Pup weights at PND 0, 14 and 21 were significantly decreased at 301 to 923 mg/kg/d.
Waterman et al., 2000	(BAuA, 2001; NICNAS, 2012; NTP, 2003; United States Environmental Protection Agency, 2022)	SD rats (30/sed/group) 2-gen study	0.0, 0.2, 0.4, or 0.8 % (0, 114–395, 235–758, 467–1541 mg/kg bw/d)	Diet, 10 weeks before mating and for an additional 7 weeks, through mating, gestation, and lactation	LOAEL (development): 0.2 % (143-285 mg/kg bw/d)	Significantly reduced weight gain observed in F1 pups at PND 21 at 0.2 %
Zacharewski 1998	(NTP, 2003)	SD rats (10/group) (ovariectomized)	0, 20, 200, 2000	Gavage 4 days (no exact period given)	NOAEL: 2000	No increases in uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats treated up to 2000 mg/kg DINP Statistically significant decreases in uterine wet weight were observed in the 2000 mg/kg group

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Tardif et al., 2023)		SD rats (expressing GFP specifically in germ cells)	10 µM	Three days	NOAEC 10 µM	Testicular cells collected at GD 15 were grown ex vivo no significant effects on testosterone release, luteinising hormone-stimulated testosterone, Leydig cell density and area, germ cell density or Sertoli cell proliferation less toxicity to foetal testis development than other plasticisers such as DEHP

Table VI.45: Overview of studies on the effect of DINP on gene activation, expression, enzyme activity and further effects

Reference	Cited in	Findings
BIBRA 1985; reviewed by EC JRC 2003	(California EPA et al., 2013)	- Male and female F344 rats were fed diets containing 0, 0.6, 1.2 or 2.5 % DINP for 21 days - Hepatic peroxisomes, hepatic peroxisome proliferation (assessed by electron microscopy) and PCoA activity were significantly increased compared to controls at doses of 1.2% and higher.
Bility et al. 2004	(California EPA et al., 2013)	- MINP caused a dose-dependent increase in PPARα activation at concentrations ≥ 3 µM for mouse PPARα and ≥ 10 µM for human PPARα. - MINP activated neither human nor mouse PPARβ. - MINP caused a dose-dependent increase in mouse and human PPARγ activation at concentrations of ≥ 3 µM and ≥ 30 µM, respectively. - MINP caused a dose-dependent increase in the induction of acetyl-CoA oxidase and CYP4A mRNA in FaO cells but not in HepG2 cells - A strong induction of adipogenesis was observed with 50 µM MINP
(Chiu et al. 2022)		- Influence of DINP on the microbiota, intestinal integrity and immune status of the colon was investigated in mice - Female mice (6–8-week-old CD-1 mice) were orally administered different doses of DINP (20 µg/kg, 200 µg/kg, 2 mg/kg, 20 mg/kg and 200 mg/kg) for 10-14 consecutive days - After treatment, the mice were euthanised, colonic contents were analysed with 16S rRNA gene sequencing to identify the bacteria in the colonic contents - Environmentally relevant dose of 200 µg/kg significantly increased a Lachnoclostridium *taxon* and decreased *Blautia* compared to the control - Overall, minimal changes were observed in the colon microbiota - Three anaerobic bacterial strains were identified from the colon using DINP as their sole carbon source - DINP exposure did not alter the protein levels of IL-6, TNF alpha, claudin-1 and mucin-1 - The results of the study show that DINP exposure alters the gut microbiota and that the gut contains DINP-degrading microbes
Deng et al. 2019	(United States Environmental Protection Agency, 2022)	- Exposure to DINP induced an increase in blood pressure through activation of the ACE/ AT1R axis and inhibition of NO production
(Eljezi et al. 2017)		- L929 cells were exposed to DINP and Monoisononylphthalate (MINP, primary metabolite of DINP) daily at concentrations of 0.01, 0.05, and 0.1 mg/mL - MTT assays and quantification of the viability percentage was performed every day - Reduced cell proliferation (40%) was only seen at the highest concentration of 0.1 mg/mL for DINP exposure

Reference	Cited in	Findings
		- For MINP, a concentration-dependent decrease in viability was observed. While weakly toxic at 0.05 mg/mL, cell viability fell sharply at 0.1 mg/mL reaching up to 80% cell death
(Eljezi et al. 2019)		- L929 cell lines were exposed daily to secondary metabolites of DINP at 0.01, 0.05, and 0.1 mg/mL for seven days. - The secondary metabolites 7-cx-MMeOP, 7-OH-MMeOP, and 7-oxo-MMeOP did not show any signs of significantly increased cytotoxicity against the DMSO control test
Huang et al. 2019	(United States Environmental Protection Agency, 2022)	- DEHP and DINP induce tissue- and gender-specific disturbances in fatty acid and lipidomic profiles in neonatal mice
Hüls 1992; reviewed by EC JRC 2003	(California EPA et al., 2013)	- Female F344 rats were given DINP at doses of 0, 25, 75, 150 or 1500 mg/kg-day by gavage for 14 days. - DINP significantly induced hepatic LCoA activity in the highest dose group.
Hwang et al. 2017	(United States Environmental Protection Agency, 2022)	- Effects of diisononyl phthalate on osteopenia in intact mice - DINP contributes osteopenia by the destruction of the microarchitecture and enhancement of osteoclast activity
Imai et al. 2006	(United States Environmental Protection Agency, 2022)	- Effects of phthalate esters on the sensitization phase of contact hypersensitivity induced by fluorescein isothiocyanate - DINP showed no significant increase in the FITC-positive cell number
Kang et al. 2016	(United States Environmental Protection Agency, 2022)	- Diisononyl phthalate aggravates allergic dermatitis by activation of NF-kB - DINP aggravates allergic-dermatitis-like lesions
Kaufmann et al. 2002	(California EPA et al., 2013; United States Environmental Protection Agency, 2022)	- Male and female B6C3F1 mice were fed diets containing 500, 1,500, 4,000 or 8,000 ppm DINP for 4 weeks. - Both male and female mice exhibited significantly increased hepatic peroxisome proliferation compared to controls at doses of 1,500 ppm and higher. Hepatic PCoA activity was significantly increased in male and female mice compared to controls at doses of 500 and 1,500 ppm, respectively.
Koike et al. 2010	(United States Environmental Protection Agency, 2022)	- DINP on atopic dermatitis *in vivo* and immunologic responses *in vitro* - DINP aggravated AD-like skin lesions related to Dp - DINP enhanced the expression of cell surface activation markers and interleukin- 4 production
(Krause et al. 2023)		- Altered gene expression *in vitro* on mucosa-associated invariant T cells (MAIT) was investigated - A very high final concentration of 2536.65 µM was used, with incubation for 24 hours - No significant effects on MAIT cells themselves, but the authors observed a slight but significant modulation of the gene expression of the cells after exposure
Lington et al. 1997	(California EPA et al., 2013)	- In a two-year DINP feeding study in male and female F344 rats, slight hypertrophy of liver parenchyma cells was observed at the

Reference	Cited in	Findings
		high dose (6,000 ppm) after 2 years. Ultrastructural examination of the livers of these animals did not reveal any treatment-induced peroxisome proliferation.
Ma et al. 2015	(United States Environmental Protection Agency, 2022)	- Cognitive deficits and anxiety induced by DINP in mice - Histopathological alterations in the brain and increased levels of oxidative stress, and inflammation
Moore 1998a; reviewed by CPSC 2001	(California EPA et al., 2013)	- In a two-year DINP feeding study in male and female F344 rats, liver hypertrophy and PCoA activity were significantly increased at 2, 13, 79 and 104 weeks in male and female animals fed high doses (12,000 ppm).
Moore 1998b; reviewed by CPSC 2001	(California EPA et al., 2013)	- In a two-year DINP feeding study in male and female B6C3F1 mice, a significant increase in hepatic PCoA activity was observed in both male and female animals after 78 or 104 weeks of exposure to the highest dose used (8,000 ppm). Hepatic PCoA activity was not studied at the lower doses.
Neier et al. 2019	(United States Environmental Protection Agency, 2022)	- Perinatal exposures to phthalates and intracisternal A-particle (IAP) DNA methylation in weanling mice - DNA methylation and changes in sex specific effects were observed
Peng 2015	(United States Environmental Protection Agency, 2022)	- Ice brain tissue injury induced by DINP exposure and the protective application of vitamin E - DINP induced brain damage, and oxidative stress, inflammation, and apoptosis
Roy et al., 2004 and Takeuchi et al., 2005)	(United States Environmental Protection Agency, 2022)	- DINP did not activate or inhibit AhR activity or AR activity up to 1×10^{-10} to 1×10^{-4} M tested - DINP did not show any estrogenic/anti-estrogenic or androgenic/anti-androgenic activity up to concentrations of 10^{-5} M
Sadakane et al. 2014	(United States Environmental Protection Agency, 2022)	- DINP on atopic dermatitis in NC/Nga mice - DINP at doses lower than the NOAEL tends to increase the allergic response in animal AD models, but only DINP administration slightly aggravates allergen-induced ADSL production
Sedha et al. 2015	(United States Environmental Protection Agency, 2022)	- *In vivo* estrogenic potential of DINP in rats - DINP is unable to induce elevation in the uterine weight and unable to cause vaginal opening indicating non-estrogenic potential of DINP
(Sheikh et al. 2016)		- The binding activity of DINP to amino acid residues of sex hormone binding globulin SHGB was determined using in silico models - SHBG is a plasma carrier protein that binds androgens and oestrogens and is a potential target for the function of phthalates as endocrine disruptors in the body - Longer chain phthalates such as DINP showed lower binding affinity in the model - The study is only of theoretical value, as no exposures were mentioned

Reference	Cited in	Findings
Smith et al. 2000	(California EPA et al., 2013)	- Male F344 rats and male B6C3F1 mice were exposed to DINP in the diet for 2 or 4 weeks. Doses were 0, 1000 and 12,000 ppm, and 0, 500 and 6000 ppm for rats and mice, respectively. - DINP significantly increased hepatic PCoA activity in rats and mice compared to controls after 2 or 4 weeks of treatment with the high dose but not with the low dose.
(Song et al. 2021)		- Male Kunming mice were exposed to 2, 20, and 200 mg/kg/d DINP, 100 mg/kg/d vitamin E, light + 2, 20, and 200 mg/kg/d DINP, and light + 200 mg/kg/d DINP + 100 mg/kg/d vitamin E orally for 28 days - The group exposed to continuous light and 200 mg/kg/d DINP showed the slowest decrease in escape latency during a Morris water maze test - Increasing DINP concentrations increased randomness of the swimming trajectories of the mice and further increased the degree of damage done to the vertebral body cells in the hippocampal CA 3 area, as cells became more swollen and rounder, and intercellular space expanded and cells were arranged more loosely and in a disorderly way - Increased DINP concentrations further promoted a gradual decrease in anti-apoptotic proteins in the cells and an increase in pro-apoptotic protein in the cells
(Taş et al. 2019)		- DINP was tested *in vitro* at concentrations of 1, 10, 100 and 1000 µM on A549, H292, and BEAS-2B cells - Phospho-NF-κB level, as well as the phospho-STAT3 level in A549 and BEAS-2B cells increased - According to the authors, the results indicate induced inflammatory responses, cell permeability and malignant transformation in lung epithelial cells through activation of the oncogenic and inflammatory transcription factors STAT3 and NF-κB
(Tekin et al. 2020)		- Primary canine testicular parenchymal cells were exposure *in vitro* for 24 h between concentrations of 0.001 and 2.5 nM to determine the cytotoxic effects of DINP and other phthalates - Higher molecular weight phthalates such as DINP appeared to have more severe effects than lower molecular weight phthalates - for DINP, an IC50 of 57.69 ± 7.97 nM was determined
Valles et al. 2003	(California EPA et al., 2013; United States Environmental Protection Agency, 2022)	- Male and female B6C3F1, SV 129 and SV129 PPARα-null mice (26-30 weeks old) were exposed to 0 or 8,000 ppm DINP in the diet for one or three weeks. Male mice were exposed to DINP and female mice were exposed to DINP (CAS number 68515-48-0). - Increases in LBWR were observed in male and female B6C3F1 and SV129 wild-type mice and in female PPARαnull mice exposed to DINP. No increase in LBWR was observed in DINP-exposed male PPARα-null mice. - These data suggest that some hepatic effects, such as increased LBWR, thought to be dependent on PPARα activation alone, may occur independently of PPARα activation. These results also suggest that responses to DINP depend on the life stage of exposure.

Reference	Cited in	Findings
(Wang et al. 2023)		- In an *in vitro* experiment, porcine oocytes were exposed to DINP concentrations of 0, 25, 50 or 100 µM by adding the substance to a maturation medium - The cells were then incubated in the medium for 44 h - DINP exposure impaired *in vitro* maturation of oocytes by disrupting cytoskeletal organisation, CG distribution and mitochondrial dynamics, leading to defects in sperm binding, fusion and fertilisation caused by excessive ROS-induced apoptosis. - DINP exposure weakened oocyte meiotic competence (78.9% vs. 53.6%, $P < 0.001$) and fertilisation ability (78.5% vs. 34.1%, $P < 0.0001$) during *in vitro* maturation - The values compiled in the abstract could not be derived from the results and were often not accompanied by units, which is why no greater significance should be attached to the study
Wu et al. 2015	(United States Environmental Protection Agency, 2022)	- Long-term dermal exposure to DINP exacerbates atopic dermatitis through oxidative stress - High doses of DINP may aggravate atopic dermatitis
(Yurdakok-Dikmen et al. 2019)		- Cultures of bovine primary male reproductive cells (n = 3) and spermatozoa (n = 4) were exposed to different phthalates to determine cytotoxicity - The IC50 of DINP on epididymal head cells was 0.02 - 0.176 ng/ml, and on epididymal body cells 0.003 ng/ml - The IC50 of parenchymal cells of the testis was 0.207 ng/ml, that of the mediastinum 0.009 ng/ml and of sperm cells 0.550 ng/ml - No test concentrations used for DINP were listed in the study
(Hwang, Paik, and Yee 2017)		- $CD4^+$ T cells were isolated from the spleens (species unknown) and exposed to DINP at 1–1000 µM without exact concentration or duration given - No cytotoxic effects at any concentration - DINP was shown to suppress the polarization of Th1 and enhance the polarization of Th2 from naïve $CD4^+$ T cells *in vitro* - DINP induced allergic asthma via the production of IL-4, IL-5, IgE and IgG1 and the reduction of IFN-γ and IgG2a - The effects described cannot be followed up, the description of the study is very poor
(Zhang et al. 2019)		- *In vitro* 10-day exposure of 3T3-L1 preadipocytes to DINP (100 µM) induced adipogenesis adipocytes as shown by lipid accumulation and gene expression of adipogenic markers - Expression of peroxisome proliferator-activated receptor-gamma (PPARγ), CCAAT/enhancer-binding protein alpha (C/EBPα) and C/EBPβ was upregulated while the expression of sterol regulatory element binding transcription factor 1 (SREBF1) and C/EBPδ was not affected - DINP-induced adipogenesis could be inhibited by using the selective PPARγ antagonist GW9662

Reference	Cited in	Findings
		- 1181 differentially expressed genes (640 genes were upregulated, 541 genes were downregulated) were detected in 3T3-L1 preadipocytes exposed to 50 mM DINP by RNA seq analysis
(Poitou et al. 2022)		- In *vitro* investigation of DINP on cell viability and mitochondrial metabolism of endothelial cells (HMEC-1) - Treated with a concentration of 15 mg/L DINP. (40 µM) for 24 to 72 hours - Initially, an increase was observed at 24 h for DINP (115 ± 7%), followed by a decreasing cell viability for 48 and 72 h - Decreased basal oxygen consumption rate, reserve capacity and ATP production were observed after DINP exposure
(Gutiérrez-García, Choudhury, and De Leon-Rodriguez 2019)		- HepG2 cells were exposed to 0.1, 1, 10, and 100 µg/mL for 48 hours to quantify for gene expression and for 72 hours to analyse protein expression of Sirt1, Sirt2, Sirt3, Sirt5 and acetylated proteins - Concentration dependent in cell viability observed, as 0.1 and 1 µg/mL showed insignificant levels of cell death compared to ctrl, but dosage levels of 10 and 100 µg/mL lead to 15% decrease - Gene expression was affected by DINP exposition for Sirt1, Sirt2, Sirt3, and Sirt5. Sirt1, Sirt2, and Sirt5 decreased by 50% at 1 and 10 µg/mL, decreasing further to 75% total decrease at 100 µg/mL - Sirt3 expression already decreased by 50% at 0.1 µg/mL but was not decreasing further with increasing concentrations - 25% decrease in expression of Sirt1 at 1 µg/mL, up to a 50% decrease at 100 µg/mL. Sirt3 also showed a decrease up to 50% at 100 µg/mL of DINP exposure - Protein acetylation levels were doubled at 100 µg/mL DINP exposure

The proposed PPARα liver tumour MOA hypothesis identifies increased hepatic cell proliferation and disruption of apoptosis as PPARα-dependent causal events leading to tumour formation. However, data on short-term induction of hepatocellular proliferation in DINP-exposed B6C3F1 mice are conflicting, and no long-term hepatocellular proliferation has been demonstrated in DINP-exposed rats (California EPA et al., 2013). These studies are listed Table IX.85.

Table VI.46: Overview of studies on cell proliferation

Reference	Cited in	Findings
Moore 1998a; reviewed by CPSC 2001	(California EPA et al., 2013)	- In the feeding study in male and female F344 rats, a significant increase in mitotic cell count and mean hepatocyte labelling index was observed in the livers of all 5 male and 5 female high-dose animals after 1 week of treatment. - After 2, 13 and 79 weeks of treatment, the number of mitotic cells and the marker indices in the liver were no longer increased compared to controls. Therefore, the initial increase in liver cell proliferation in these animals was not sustained, despite the persistent increase in markers of peroxisome proliferation increased hepatic hypertrophy and PCoA activity.
Moore 1998b; reviewed by CPSC 2001	(California EPA et al., 2013)	- In the feeding study in male and female B6C3F1 mice, there was no evidence of increased liver cell proliferation as measured by the mean labelling index at a dose of 8,000 ppm after 78 or 104 weeks of treatment compared to controls.
Smith et al. 2000	(California EPA et al., 2013)	- 12,000 ppm DINP in the diet induced periportal parenchymal cell proliferation in male F344 rats compared to controls after two weeks of exposure (measured by increase in labelling index). After four weeks of exposure, hepatocellular proliferation was not increased in the 12,000 ppm DINP males compared to controls - Observation of hepatocellular proliferation in male B6C3F1 mice exposed to 6,000 ppm DINP in the diet after two weeks of exposure, but not after four weeks of exposure.
Kaufmann et al. 2002	(California EPA et al., 2013)	- Observation of significantly increased hepatocellular proliferation in liver zone 1 (portal tract) of male B6C3F1 mice compared to controls after four weeks of dietary DINP exposure at exposure levels of 1,500 ppm (282 % increase) and 8,000 ppm (1,009 % increase), but not at 4,000 ppm. However, hepatocellular proliferation was not significantly increased in liver zone 1 of female mice after four weeks of DINP exposure at any concentration. - To study apoptosis (measured by TUNEL staining) in male and female B6C3F1 mice exposed to DINP in the diet at doses of 500, 1,500, 4,000 or 8,000 ppm for either one or four weeks. Concurrent controls were performed for the four-week exposure groups but not for the one-week exposure groups, making interpretation of the results from the one-week exposure groups problematic. Rates of hepatocellular apoptosis (assessed in zones 1, 2 and 3) were not significantly different in males and females at all DINP dose levels after four weeks of DINP exposure compared to controls.
Shaw et al. 2002	(California EPA et al., 2013)	- In isolated primary F344 rat hepatocytes exposed in *vitro to* the DINP metabolite MINP at concentrations of 150 to 250 µM, DNA replication synthesis was significantly induced and transforming growth factor β1-induced apoptosis was significantly suppressed.

Reference	Cited in	Findings
Valles et al. 2003	(California EPA et al., 2013)	- Female B6C3F1, SV129 wild-type and SV129-PPARα-null mice were fed diets containing 0 or 8,000 ppm DINP for one week. - DINP treatment significantly increased hepatocellular proliferation in liver zones 1 (periportal area) and 3 (centrilobular area) of B6C3F1 females and in liver zone 3 of SV129 wild-type females compared to controls. One-week exposure to DINP did not increase hepatocellular proliferation in SV129 PPARα-null females.

Part VII

Substance monograph aluminium and its inorganic compounds and derivation of HBM guidance values (HBM-GVs) for the general population and for workers

Technical reference

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Contributing Participants	Manca Ahačič (NIJZ), manca.ahacic@nijz.si Lucija Perharič (NIJZ), lucija.perharic@nijz.si
Responsible author(s)	Maria Tannous (ANSES) : maria.tannous@anses.fr Keyvin Darney (ANSES): Keyvin.darney@anses.fr
Co-authors	/
Internal Reviewers	WP4 co-leaders
External Reviewers	Experts from Finnish NH, German NH, Swedish NH, UK NH Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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1 PU = Public

PP = Restricted to other programme participants (including the Commission Services)

RE = Restricted to a group specified by the consortium (including the Commission Services)

CO = Confidential, only for members of the consortium (including the Commission Services)

2 WP/task co-leader(s) from the same WP

3 Independent Reviewers that should not be part of the task related to the AD/D or part of projects related to the Task

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Version	Date	Reviewer name/ Institutions	Short description of changes
Draft version	21/06/2024	ANSES	First report version shared for consultation
Consultation phase (National Hubs and project partners)	23/07/2024 - 17/09/2024	Partners: Manca Ahačič (NIJZ), Lucija Perharič (NIJZ) Experts from Finnish NH, German NH, Swedish NH, UK NH	See Annex of Deliverable AD 4.3
Revised version by ANSES	23/10/2024	For approval by P4.1.3.1.a partners	-
Final version	31/01/2025		
AD 4.3 Reviewer comments	24/04/2025	PARC Reviewer: EAA (AT)	Comment 1: I do understand the rationale for selecting the NOAEL from the occupational study for the HBMGV for workers. However, as there are concerns related to aluminium exposure and several neurogenerative diseases I would strongly question the inter-individual uncertainty factor of 2.
			Comment 2: Sensitive population: I think they are not covered by this assessment: there is no chapter or paragraph on sensitive populations. Even the word 'sensitive' appears only twice in the document. Vulnerable subgroups of the population include e.g. children and patients.
			Comment 3: What I think is missing or at least should be mentioned here is that consumer uses are highly relevant for exposure of the general population (and workers as well). According to the BfR food already contributes to ~ 50% of the TWI. Food contact materials and food additives are relevant and cosmetics can contribute considerably. Please see DOI 10.17590/20191115-135258, from 2019
			Comment 4: Regarding the inter-species uncertainty factor (UFA) of 1, there might be considerable differences between high (occupational) and lower exposures also in terms of e.g. disruption of homeostasis of metals such as magnesium, calcium, and iron.
			Comment 5: Regarding the quality of the database uncertainty factor (UFD)of 1, in my view the database is considerable, however there are data gaps related to specific concerns about aluminium exposure and certain health outcomes as well as vulnerable populations (aluminium crosses the placental barrier and the developing brain is presumably more sensitive).

Version	Date	Reviewer name/ Institutions	Short description of changes
Final version	29/04/2025	ANSES	Response to Comment 1: Neurodegenerative diseases and aluminium exposure are discussed in chapter 4.8.1.1.1. Limitation of epidemiological studies are presented. These studies mainly investigate Alzheimer's disease for which both EFSA and WHO concluded that aluminium does not constitute a risk factor. The reasoning behind the UF of 2 for the general population is the difference in variability between workers and the general population. Default UF to account for interindividual variability are of 5 and 10 for workers and the general population respectively. Thus, we consider a ratio of 2 between these 2 populations. Moreover, the UF for interindividual variability applied to derive the workers' HBM-GV is of 1 "because the key study corresponds to the target population". Therefore, there is no justification to increase the UF for interindividual variability above 2 for the general population.
			Response to Comment 2: The section on sensitive population is available in chapter 4.12 and involve people suffering of kidney disease.
			Response to Comment 3: A paragraph has been added in sub-chapter 3.4 covering human exposures.
			Response to Comment 4: The UFA was not changed. The inter-species uncertainty factor is not related to the exposure level, also this UF only applies when the point of departure come from animal study which is not the case here.
			Response to Comment 5: The UFD was not increased. We acknowledge these data gaps; however, the quality of the overall database was considered sufficient regarding the selected critical effect in human and does not require to increase the related UFD.

"Funded by the European Union. Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them."

Abstract

Human biomonitoring guidance values (HBM-GVs) have been derived for aluminium and its inorganic compounds, for the general population and workers, and are described in this report.
An update of the toxicological profile, based on an extensive literature search, was carried out in order to proceed with the derivation of these HBM-GVs. Urinary aluminium was considered to be the most relevant biomarker of aluminium exposure, as it is highly sensitive to small changes in external exposure, and a good association was found between this biomarker and health effects in humans. Cognitive impairment was chosen as the critical effect. This effect has been observed in numerous epidemiological studies of workers. The longitudinal study conducted by Buchta et al. (2003) and Kiesswetter et al. (2009), which included over 90 aluminium welders in the car-body construction industry and 50 controls, showing no difference in neurological symptoms at a urinary level of 37.87 µg/g creatinine was selected as the key study.

The NOAEL of 37.87 µg/g creatinine rounded up to 40 µg/g creatinine was retained as the point of departure (PoD) for deriving both of the HBM-GVs. For workers, no uncertainty factor was applied to this PoD, but for the general population, an inter-individual uncertainty factor of 2 was applied as the key study was conducted in a population of workers (ratio between the default inter-individual uncertainty factors of 10 and 5 in the general population and workers, respectively). This leads to an $HBM\text{-}GV_{Workers}$ of 40 µg/g creatinine and an $HBM\text{-}GV_{GeneralPopulation}$ of 20 µg/g creatinine. Sampling time is not critical for the general population chronically exposed to aluminium, but to avoid contamination and ensure good reproducibility, it is advisable to take samples in the morning, upon waking or after showering. For workers, samples taken at the end of a shift, after several shifts, are recommended.

It should be noted that, for the general population, the biomonitoring equivalent concentrations based on the oral toxicological reference value of JECFA (2012) using the mass balance approach or the 3-compartment kinetic model of Poddalgoda et al. (2021), were much higher than the derived, more conservative, HBM-GV, and were therefore not retained.

Key Words

Human biomonitoring, human biomonitoring guidance values, HBM-GV, aluminium, workers, general population, biomarker of exposure

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Authors and Acknowledgements

Authors

Maria Tannous (ANSES): maria.tannous@anses.fr

Keyvin Darney (ANSES): Keyvin.darney@anses.fr

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Abbreviations and Acronyms

Al	Aluminium
AAS	Atomic Absorption Spectrophotometry
ANSES	Agence nationale de sécurité sanitaire alimentation environnement travail (French agency for food, environmental and occupational health and safety)
ACH	Aluminium Hexahydrate Chloride
AchE	Acetylcholinesterase
ACGIH	American Conference of Governmental Industrial Hygienists
ACP	Acid phosphatase
Afssaps	The old name of the French National Agency for the Safety of Medicines and Health Products (ANSM)
ALP	Alkaline phosphatase
AOR	Adjusted Odds Ratio
ATIME	Average Reaction Time
ATSDR	Agency for Toxic Substances and Disease Registry
ATP	Adenosine triphosphate
ATW	Aluminium-treated drinking water
AVLT	Auditory Verbal Learning Test
BDI	Beck depression inventory
BME	Biomarker of exposure
BMI	Body mass index
BMP-2	Bone morphogenetic protein 2
BNDF	Brain Derived Neurotrophic Factor
bw	Body weight
cAMP	Cyclic adenosine monophosphate
CDC	US Centers for Disease Control and Prevention
CDT	Clock Drawing Test
CES	Expert Committee (Comité d'experts spécialisé)
CFU-E	Colony-forming units-erythroid
CLP	Classification, Labelling and Packaging
CNS	Central Nervous System
Col II	Type II collagen
Cu	Copper
CX3CL1	Fractalkine
DFG	Deutsche Forschungsgemeinschaft (German research foundation)
DGS	Directorate General for Health
DMN	Default mode network
DSBT	Digit Span Test backward
DSFT	Digit Span Test forward

DST	Digit Span Test
ETAAS	Electrothermal Atomic Absorption Spectrometry
ECHA	European chemicals agency
EEG	Electroencephalography
EFSA	European Food Safety Authority
ERP	Event-related potentials
EURO-NES	European neurobehavioral evaluation system
F1	First generation bred from a pair of parents
Fe	Iron
FOME	Fuld Object Memory Evaluation
FSH	Follicle-stimulating hormone
GerES	German Environmental Survey
Gd	Gestational day
GFAAS	Graphite furnace atomic absorption spectrometry
GFA-P	Glial fibrillary acidic protein
GHS	Globally Harmonized System
GSH-PX	Glutathione peroxidase
HBM4EU	European Human Biomonitoring Initiative
HBM-GV	Human biomonitoring guidance values
HCSP	Haut Conseil de la Santé Publique (French High Council for Public Health)
HRV	Health reference value
HSL	Health and Safety Laboratory (Buxton, Derbyshire, UK)
IARC	International Agency for Research on Cancer
CI	Confidence Interval
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
INERIS	Institut national de l'environnement industriel et des risques (French National Institute for Industrial Environment and Risks)
INRS	Institut national de recherche et de sécurité (Reference body for occupational risk prevention in France)
INSPQ	Institut national de santé publique du Québec
ip	Intraperitoneal injection
IQR	interquartile range
IV	Intravenous
Ld	Lactation day
LD50	Median lethal dose
LDH	Lactate dehydrogenase
LDH-x	Lactate dehydrogenase isoenzyme
LH	Luteinizing hormone
LLNA	Local lymph node assay

LMW	Low molecular weight proteins
LOAEL	Lowest Observed Adverse Effect Level
LOD	Level of detection
LOQ	Limit of quantification
MAK Commission	German Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area
MCH II	Major histocompatibility complex class II
MCI	Mild cognitive impairment
MCT	Monocarboxylate transporter
MMF	Macrophagic myofasciitis
MMSE	Mini-Mental State Examination
MMSE-AE	Mini-Mental State Examination adjusted for age and education
MoCA	Montreal cognitive assessment Basic
N/A	Not applicable
NCTB	Neurobehavioral Core Test Battery
NHANES	National Health and Nutrition Examination Survey
NPC	Nonparametric test combination
NIOSH	National institute for occupational safety and health
NMU	N-nitroso-N-methylurea
NOAEL	No Observed Adverse Effect Level
NS	Not specified
OEHHA	Office of Environmental Health Hazard Assessment (Californie - États-Unis)
OEL	Occupational exposure limit
OR	Odds ratio
OSHA	Occupational Safety and Health Administration
PAHs	Polycyclic aromatic hydrocarbons
P-Al	Aluminium levels in plasma
PARC	Partnership for the Assessment of Risks from Chemicals
PBK	Physiological-based toxicokinetic
PND	Post Natal Day
PNR	Post-rotatory nystagmus
pRfD	Provisional oral reference dose
pRfC	Provisional inhalation reference concentration
PoD	Point of departure
POMS	Profile of mood states questionnaire
PPM	Parts per million
PSQI	Pittsburg sleep quality index
PSS14	Perceived stress scale
QSM	Quantitative Susceptibility Mapping

REACh	Regulation (EC) No 1907/206 of 18/12/06 concerning the registration, evaluation, and authorisation of chemicals
RT	Reaction time
S-Al	Aluminium levels in serum
SCCS	Scientific Committee on Consumer Safety
SDH	Succinate dehydrogenase
SEM	Standard Error of the Mean
SOD	Superoxide dismutase
SPF	Santé Publique France (French Public Health Agency)
SPM	Standard Progressive Matrices test
SRT	Simple reaction time
SST	Static Steadiness Test
TGF-b1	Transforming growth factor b1
TNO	Netherlands organisation for applied scientific research
TRV	Toxicological reference value
TWA	Time weighted average
TWI	Tolerable weekly intake health-based guidance value
U-Al	Aluminium levels in urine
UF	Uncertainty factor
UFA	Uncertainty factor for interspecies variability
UFD	Database uncertainty factor
UFH	Uncertainty factor for inter-individual variability
UFL	Uncertainty factor for using a LOAEL instead of a NOAEL
UFs	Uncertainty factor for sub-chronic to chronic transposition
US EPA	United States Environmental Protection Agency (États-Unis)
WG	Working group
VFT	Verbal Fluency Test
WAIS	Wechsler adult intelligence scale
WMS	Wechsler memory scale
Zn	Zinc

1 Introduction

Under the European Partnership for the Assessment of Risks from Chemicals (PARC), Human Biomonitoring Guidance Values (HBM-GVs) are recommended for the general population and workers. These values are proposed for priority substances, mainly those included in the Aligned Studies, representing the biomonitoring programme of this partnership.

Aluminium is among these priority substances; therefore, the aim of this deliverable is to derive HBM-GVs for aluminium and its inorganic compounds in order to assess human health risks on the basis of results from the biomonitoring study.

2 Methods

To derive HBM-GVs, ANSES relies on its TRV derivation guide (ANSES, to be released). The derivation process involves:

- Collecting data useful for characterizing the chemical agent (identification, physicochemical properties, classifications), as well as general information on uses, sources, and exposures.
- Drawing up a toxicological profile to define the effects observed in humans and animals, linked to the different types of exposure to the chemical agent, characterised by their duration and route of exposure (oral, respiratory, dermal), as well as the sensitive populations. Any beneficial effects of the chemical agent are not described in the toxicological profile.
- Selecting the most relevant biomarker of exposure on the basis of several criteria (e.g., the existence of a relationship between the concentration of this biomarker and health effects and/or external concentrations or doses, an essential criterion; its elimination half-life; its specificity; the availability of analytical methods; etc.).
- Selecting the most sensitive adverse effect.
- Choosing the derivation approach according to whether or not the selected critical adverse effect has a threshold.
- Selecting the key study. Good-quality human studies are preferred over animal studies and predictive methods.
- Allometric or temporal adjustments, if necessary.
- Adding uncertainty factors to derive the HBM-GV, whether for the general population or workers.

To this end, the synthesis of toxicity data following the exposure to aluminium and its inorganic compounds was written based on synthesis reports produced by ATSDR (ATSDR, 2008), EFSA (EFSA, 2008), ACGIH (ACGIH, 2008), JECFA (JECFA, 2012), DFG (DFG, 2013 and 2019) and SCCS (SCCS, 2014, 2019, 2020, 2022 and 2023), supplemented by a literature review.

This literature search was conducted using the PubMed and Scopus databases between 2007 and July 2023 based on the following keywords: “Aluminium”, “aluminium compound*”, “toxicity”, “toxicokinetic”, “health effect*”.

3 General characteristics

3.1 Substance identification

Aluminium (Al) is a silver-coloured, low-density, malleable and ductile metal (INRS 2021). It is a ubiquitous metallic element which is the most widespread in the earth's crust with 8% Al (INERIS 2015), mainly as a silicate. There are many compounds of aluminium. A non-exhaustive list below presents the compounds most commonly described in the literature (Table VII.47Table VII.47).

Table VII.47: General information on the most commonly described aluminium compounds (INERIS 2005; 2015)

Name	CAS Number	EC Number	Physical state	Molecular weight (g/mol) [23]
Aluminium (Al)	7429-90-5	231-072-3	Solid	26.98
Aluminium carbonate ($Al_2(CO_3)_3$)	14455-29-9	238-440-2	White powder	233.99
Aluminium chloride ($AlCl_3$)	7446-70-0	231-208-1	Solid	133.34
Aluminium chloride, basic ($Al_2(OH)_nCl_{6-n}$)	1327-41-9	215-477-2	Aqueous solution	
[Aluminium chlorohydrate / Aluminium chloride hydroxide when n=5 ($Al_2Cl(OH)_5$)]	[12042-91-0]	[234-933-1]	[Crystallised solid when n=5]	174.45
Aluminium citrate ($C_6H_5AlO_7$)	31142-56-0	250-484-4	White crystallised solid	216.08
Aluminium fluoride (AlF_3)	7784-18-1	232-051-1	Solid	83.98
Aluminium hydroxide (AlH_3O_3)	21645-51-2	244-492-7	Crystallised solid or amorphous powder	78.00
Aluminium lactate ($C_9H_{15}AlO_9$)	18917-91-4	242-670-9	White powder	294.19
Aluminium nitrate (AlN_3O_9)	13473-90-0	236-751-8	Crystallised solid	213.00
Aluminium oxide (Al_2O_3)	1344-28-1	215-691-6	Crystalline	101.96
Aluminium oxide hydroxide, Boehmite (AlOOH)	1318-23-6	215-284-3	Crystalline solid	59.99
Aluminium orthophosphate / aluminium phosphate (AlO_4P)	7784-30-7	232-056-9	Powder	121.95
Aluminium silicate (Al_2SiO_5)	12141-46-7	235-253-8	Crystalline solid	162.05
Aluminium sodium dioxide (AlO_2Na)	1302-42-7	215-100-1	Solid	81.97
Aluminium sulphate ($Al_2O_{12}S_3$)	10043-01-3	233-135-0	Solid and Aqueous solution	342.2

[23] PubChem: https://pubchem.ncbi.nlm.nih.gov/

3.2 Physico-chemical properties

Some aluminium salts, for example, aluminium sulphate, aluminium chloride (hydrated) and aluminium nitrate, are readily soluble in water, whereas other aluminium species such as elemental aluminium, aluminium oxide, aluminium phosphate, and aluminium silicate are very sparingly soluble (Vesa Riihimäki et Aitio 2012). Actually, aluminium citrate is the most soluble and bioavailable aluminium salt (Poirier et al. 2011). The table below (Table VII.48) presents the solubility of the different aluminium salts in water and in other solvents.

Table VII.48: Solubility of aluminium salts in water and other solvents

Name of aluminium compound	Solubility in water	Solubility in other solvents
Aluminium (Al)	Insoluble in water at 20°C	Soluble in HCl, H_2SO_4, hot water, and alkalies
Aluminium carbonate ($Al_2(CO_3)_3$)	Insoluble	Soluble in hot HCl or H_2SO_4
Aluminium chloride ($AlCl_3$)	450-458 g/L at 20 °C	Soluble in benzene, carbon tetrachloride, chloroform
Aluminium chlorohydrate ($Al_2Cl(OH)_5$)	Soluble in water forming slightly turbid colloidal solutions (up to 550 g/L)	No data
Aluminium citrate ($C_6H_5AlO_7$)	Most soluble	No data
Aluminium fluoride (AlF_3)	5,59 g/L at 25 °C	Sparingly soluble in acids and alkalies; insoluble in alcohol and acetone
Aluminium hydroxide ($Al(OH)_3$)	Insoluble in water at 20°C	Soluble in alkaline or acid solutions
Aluminium lactate ($C_9H_{15}AlO_9$)	Freely soluble in water: 206 g/L at 25 °C	No data
Aluminium nitrate (AlN_3O_9)	Very soluble in water: 637 g/L at 25 °C	Very soluble in alcohol; very slightly soluble in acetone; almost insoluble in ethyl acetate, pyridine
Aluminium oxide (Al_2O_3)	Soluble in cold water, 0.000098 g/100 cc; insoluble in hot water	Very slightly soluble in acid, alkali
Aluminium oxide hydroxide (AlO(OH))	No data	No data
Aluminium phosphate (AlO_4P)	Practically insoluble in water	Practically insoluble in acetic acid; very slightly soluble in concentrated HCl and HNO_3 acids
Aluminium silicate (Al_2SiO_5)	Insoluble in water	Insoluble in organic solvents
Aluminium sodium dioxide (AlO_2Na)	No data	No data
Aluminium sulphate ($Al_2O_{12}S_3$)	Soluble in water: 360 - 364 g/L at 20 °C	Insoluble in ethanol

Data from: Krewski et al. (2007); ATSDR 2008; Poirier et al. (2011); INRS 2021

As mentioned in Table VII.48, elemental aluminium is insoluble in water. At acidic pH, below 4, the dominant speciation of aluminium corresponds to its only oxidation state Al^{3+}, generally in the form of the hydrated complex $Al(H_2O)_6^{3+}$. At a pH between 5 and 6, the species $Al_2(OH)_2^{4+}$ and $Al(OH)_5^{2-}$ dominate. The insoluble form $Al(OH)_3$ is predominant in the pH range between 5.2 and 8.8. Above a pH of 9, the soluble species $Al(OH)_4^-$ is dominant. It is the only species present at pH above 10. Because of the behaviour of its hydroxycomplexes, aluminium is considered amphoteric (INERIS 2005).

3.3 Classification

Elemental aluminium and some of its compounds are subject to harmonized classification under Regulation (EC) No 1272/2008 (CLP). Among the aluminium compounds included in our scope of research (see Table VII.47), a harmonised classification is provided only for aluminium powder (pyrophoric and stabilized) and for anhydrous aluminium chloride (Table VII.49).

Table VII.49: Harmonised classification according to Annex VI of the EU Regulation (EC) No 1272/2008[24]

Name	CAS No	Classification		Labelling	
		Hazard Class & Category code(s)	Hazard statement code(s)	Pictogram, Signal Word code(s)	Hazard statement code(s)
Aluminium powder (Pyrophoric)	7429-90-5	Flam. Sol. 1 Water-react. 2	H250 [1] H261 [2]	GHS02 Dgr	H250 [1] H261 [2]
Aluminium powder (stabilised)	7429-90-5	Flam. Sol. 1 Water-react. 2	H228 [3] H261 [2]	GHS02 Dgr	H261 [2] H228 [3]
Aluminium chloride, anhydrous	7446-70-0	Skin Corr. 1B	H314 [4]	GHS05 Dgr	H314 [4]

[1] H250: Catches fire spontaneously if exposed to air
[2] H261: In contact with water releases flammable gas
[3] H228: Flammable solid
[4] H314: Causes severe skin burns and eye damage

According to the International Agency for Research on Cancer (IARC), there is sufficient evidence in humans for the carcinogenicity of aluminium in those exposed to production using the Söderberg process. This activity is associated with elevated incidences of cancers of bladder and lung. The cancer hazards associated with aluminium production mainly result from exposure to polycyclic aromatic hydrocarbons (PAHs) rather than from exposure to aluminium or its related compounds (INRS 2021). Samples of air emitted from aluminium smelters are mutagenic in bacteria; however, mutagenicity studies based on urine from exposed workers, as well as DNA-adducts studies of blood samples from these workers, gave equivocal results. There is sufficient evidence in experimental animals for the carcinogenicity of airborne particulate polynuclear organic matter from aluminium-production plants.

[24] https://echa.europa.eu/information-on-chemicals/cl-inventory-database (accessed 21 April 2023)

Globally, based on both experimental and human studies, there is weak-to-moderate evidence for a genotoxic mechanism underlying the effects of occupational exposures during aluminium production. IARC concluded there is sufficient evidence that occupational exposure to aluminium production is carcinogenic to humans (Group 1) (IARC 2012).

3.4 Sources and major uses

Aluminium production

In 2022, world aluminium production was 69,000,000 tons, the main producers being China (40,000,000 tons), India (4,000,000 tons), Russia (3,700,000 tons), Canada (3,000,000 tons) and the United Arab Emirates (2,700,000 tons) (USGC 2023[25]). Aluminium domestic annual production and consumption in France is around 600,000 to 700,000 tons and 1,200,000 to 1,300,000 tons, respectively[26].

First fusion aluminium metal is mainly obtained from bauxite or bauxitic laterite. Bauxite is mainly constituted of hydrated alumina, iron oxide (10-20%) and silica (about 5%). Alumina is extracted from bauxite, then transformed into primary aluminium which is refined to the desired degree of purity.

Since the end of the 19th century, the main industrial manufacturing process of aluminium has been electrolysis of alumina dissolved in a molten cryolite (Na_3AlF_6)-based hot bath (also known as the Hall-Héroult process). Alumina in its cryolite bath is introduced into a rectangular steel cell, whose carbon-coated inner walls form the cathode. A direct electric current between 100,000 and 320,000 amperes flows between the anode (either pre-baked or continuously self-baking Söderberg anode) and the cathode in a bath composed of cryolite and alumina. The electric current allows the alumina to react in contact with the carbon anode and to be transformed into aluminium and carbon dioxide which will concentrate at the top of the cell. The aluminium obtained by this method is 99.5% pure. The electrolytic cells are called pots, and the work area is called the potroom. To obtain purer aluminium, it is refined. There are two refining techniques: molten salt electrolysis and fractional crystallization. Both of these methods lead to aluminium that is at least 99.99% pure. Casting is the final step in the process where molten aluminium is poured into ingots in the foundry (Health Council of the Netherlands 2010; IARC 2012; INERIS 2015).

The nature and level of contaminants and other agents in the potroom may be influenced by the type of pot (prebake or Söderberg) and design (vertical- or horizontal-stud Söderberg), hooding and hood exhaust rate, building ventilation, size of operation and, electrical current used (cell amperage) (IARC 1984). Pre-baked anodes are produced by moulding petroleum coke and coal-tar pitch binder, which are baked at 1000-1200°C. Söderberg anodes are formed continuously from a paste of petroleum coke and coal-tar pitch. Consequently, both anodes constitute a source of exposure to carcinogenic PAHs. PAHs are formed from partial anode pyrolysis during electrolysis. In a study conducted by Konstantinov and Kuz'minykh (1971), the concentrations of benzo(a)pyrene in the prebake potrooms were found to be lower than in the Söderberg potrooms and, when pre-burned electrodes (presumably prebake) were used, benzo(a)pyrene concentrations were below the level of detection (IARC 1984).

Potential exposures to chemicals other than PAHs and aluminium reported in these occupational settings include fluorides and sulphur dioxide (skin and respiratory irritants), carbon monoxide, carbon dioxide, various trace metals (chromium, nickel, vanadium), asbestos, and also extreme heat and magnetic fields. Exposure to PAHs, fluorides and sulphur dioxide have progressively decreased over time (the late 1950s and the late 1980s), as demonstrated by atmospheric measurements and biomonitoring studies. This decrease can be explained by the progressive implementation of improved control technology; the increasing use of both collective and personal protective devices, and the increasing predominance of prebake pot-rooms (IARC 2012).

Coupled atmospheric and biomonitoring studies also demonstrate that inhalation is not the only and generally not the main route of exposure to PAH, aluminium and fluorides in the potroom, and that dermal exposure and ingestion should be taken into account.

Aluminium can be recycled multiple times without losing its original properties (lightness, conductivity, formability, durability, impermeability and multiple recyclability) and, aluminium recycled from old and new

[25] https://pubs.usgs.gov/periodicals/mcs2023/mcs2023-aluminum.pdf (accessed on May 2023)
[26] https://www.latribune.fr/entreprises-finance/industrie/industrie-lourde/souverainete-industrielle-et-energetique-le-cas-exemplaire-de-l-aluminium-francais-928225.html#:~:text=La%20production%20nationale%20actuelle%20tourne,importer%20100%25%20de%20ses%20besoins (accessed on May 2023).

scraps is equivalent to about 50% of both aluminium production and consumption. It is estimated that 75% of the aluminium produced since 1880 is still in use today. In Europe, recycling rates are over 90% in the automotive and building sectors, and 75% for aluminium cans. The aluminium recycling process requires only 5% of the energy needed to produce the primary metal[27,28].

Aluminium recycling involves 5 steps[29]:

- Collecting scrap: There are 2 categories of aluminium scrap:
- New Scrap – material that arises during the manufacture and fabrication of aluminium products, up to the point where they are sold to the final consumer.
- Old Scrap – material that has been used by the consumer and subsequently discarded (e.g.: used beverage cans, window frames, electrical cabling and car cylinder heads).
- Sorting scrap – grouping all coated aluminium together and grouping all uncoated aluminium together. Paper, plastic and other non-aluminium recycling needs to be removed.
- Crushing – by compacting the aluminium scrap, it reduces freight, storage and handling costs.
- Remelting – uncoated scrap is loaded directly into a large furnace, where it is heated at high temperatures and turned into molten form.
- Casting – molten aluminium is cast at a temperature of just over 700°C to form ingots.

Employees who process aluminium scrap might be exposed to high levels of aluminium dust during pre-processing steps that involve crushing and/or shredding and drying[30]. Thus, aluminium recycling does not involve the use of the Söderberg electrolytic refining process and therefore do not induce co-exposure to carcinogenic PAHs.

Aluminium uses

Aluminium metal and its compounds have many industrial applications (INRS 2021).

In the USA, in 2022, transportation applications (automobile, railway wagons and aircraft parts, signalling panels), accounted for 35% of domestic consumption; in descending order of consumption, the remainder was used in packaging (containers and foils), 23%; building, 16%; electrical, 10%; machinery, 7%; consumer durables, 6%; and other, 3% (U.S. Geological Survey, Mineral Commodity Summaries, January 2023)[31].

Table VII.50 presents various, non-exhaustive, applications of aluminium compounds (INERIS 2005; 2015; Krewski et al. 2007; INRS 2021) and their production in the European Union (ECHA REACH)[32].

[27] https://www.aluminium.fr/cycle-de-vie-et-recyclage/ (accessed on June 1, 2023)
[28] https://european-aluminium.eu/blog/enabling-the-circular-economy-with-aluminium/ (accessed on June 1, 2023)
[29] https://aluminium.org.au/how-aluminium-is-made/recycling-aluminium-chart/ (accessed on 1 June 2023)
[30] https://www.osha.gov/sites/default/files/publications/OSHA3348-metal-scrap-recycling.pdf (accessed on July 2023)
[31] https://pubs.usgs.gov/periodicals/mcs2021/mcs2021-aluminum.pdf (accessed on April 2023)
[32] Tonnage: Manufactured in and / or imported to the European Economic Area from: https://echa.europa.eu/fr/information-on-chemicals (accessed on April 2023)

Table VII.50: Summary of aluminium uses

Name of aluminium compound	Tonnage	Industrial uses	Uses in pharmaceuticals, food additives, cosmetics and other household products
Aluminium	≥ 10 000 000 tons per annum	Metal and alloys; foils;–in propellant and pyrotechnics. Used in various sectors: aerial electric cables, doors and windows, automobile and aircraft parts, shipbuilding and railways, signalling panels, kitchen utensils paints, explosives and fireworks.	Aluminium foil; colour ingredient; topical drugs, food packaging
Aluminium carbonate	No data	No data	Antacid; phosphate binder
Aluminium chloride	≥ 10 000 to < 100 000 tons per annum	Acid catalyst; as a hydrophobic agent for cotton impregnation, leather tanning, retention agent in paper production; flocculent and clarifying agent in water treatment	Antiperspirant
Aluminium chloride, basic	≥ 100 000 to < 1 000 000 tons per annum	No data	No data
Aluminium chlorohydrate	≥ 10 000 to < 100 000 tons per annum	No data	Antiperspirant; Astringent; anti-hyperphosphatemic
Aluminium citrate	No data	No data	Antiperspirant
Aluminium fluoride	≥ 100 000 to < 1 000 000 tons per annum	In ceramics; metallurgical flux; inhibitor of fermentation; catalyst; temperature and pH regulator in aluminium production processes; optical coatings and semiconductors	No data
Aluminium hydroxide	≥ 1 000 000 to < 10 000 000 tons per annum	Adsorbent; emulsifier; dyeing mordant; manufacture of glass; lubricants; detergents; waterproofing fabrics; flame retardant	Antacid; antidiarrheal agents; food colouring agent; topical drugs: diaper rash, antifungal; food packaging ingredient; vaccine adjuvant; opacifying agent in cosmetics and personal care products (e.g.: toothpastes)
Aluminium lactate	≥ 1 000 to < 10 000 tons per annum	Fire extinguishing foam	Antiseptic; in dental impression material
Aluminium nitrate	≥ 1 000 to < 10 000 tons per annum	Tanning agent (for leather); corrosion inhibitor; nitrating agent; extraction of uranium	Antiperspirant
Aluminium oxide	≥ 10 000 000 tons per annum.	Adsorbent; abrasive; in lubricants; water-proofing agent	No data

Name of aluminium compound	Tonnage	Industrial uses	Uses in pharmaceuticals, food additives, cosmetics and other household products
Aluminium oxide hydroxide	≥ 10 000 to < 100 000 tons per annum	No data	No data
Aluminium phosphate	≥ 1 000 tons per annum	Cement component; ceramic flux	Antacid; vaccine adjuvant; dental cement
Aluminium silicate	No data	In glass; manufacture of ceramics; semiprecious stones; enamels; paint filler	Dental cement; food packaging ingredient
Aluminium sodium dioxide	≥ 100 000 to < 1 000 000 tons per annum	Water treatment, solidification of the concrete, in the paper industry to increase the opacity, fibre retention and strength of the paper	No data
Aluminium sulphate	≥ 100 000 tons per annum	Tanning agent (leather); dyeing mordant; fire / waterproofing agent; decolorizing and clarifying agent; flocculating agent in the water purification	Antiperspirant; topical diaper; anti-fungal agent; food additive (in baking powder)

Aluminium exposure

Aluminium is ubiquitous in the environment; thus, the general population may be exposed to aluminium by the inhalation of ambient air and the ingestion of food and water (ATSDR 2008). Food is the primary common source of exposure in the general population. Indeed, aluminium can be present in food because of its natural occurrence in the environment, its leaching from food contact materials and the use of aluminium-containing food additives (JECFA 2012). Aluminium can also be used in the treatment of drinking water for coagulation and flocculation. Inhalation is likely to be, in the general population, a less important exposure pathway than is dietary exposure, but may represent a source of greater exposure in some urban environments. Furthermore, aluminium exposure can also result from the use of consumer products such as antiperspirants, cosmetics, internal analgesics (buffered aspirins), antidiarrheals, and antacids containing aluminium compounds. Some aluminium compounds are also used in vaccines as adjuvants to enhance their ability to provoke an immune response and improve the overall potency of the vaccine (ATSDR 2008). Workers are exposed to arsenic when refining the primary metal and in industries that use aluminium products (see aluminium uses in Table VII.50) as well as during welding.

4 Toxicological profile

Treatment doses given in mg/kg bw per day as reported in ATSDR 2008 had been reassessed using molecular weight of aluminium compounds (see Table VII.47).

4.1 Toxicokinetics

4.1.1 Absorption

The bioavailability of aluminium is dependent on the nature and speciation of the aluminium substance. The main mechanism of absorption is probably passive diffusion through paracellular pathways (ATSDR 2008). Results from *in vivo* uptake and *in vitro* dissolution studies show that metallic aluminium, Al_2O_3, and $Al(OH)_3$ are less bioavailable via oral and inhalation routes compared to water-soluble aluminium forms like alum ($Al_2(SO_4)_3$). Under normal physiological conditions, exposures to insoluble aluminium forms do not contribute significantly to total aluminium body burden (Willhite et al. 2021). It is however important to note that the applicability of HBM-GVs is not affected by whether soluble or insoluble compounds are involved, as they take into account what has been absorbed *in fine*, which represents the fraction responsible for toxic effects.

4.1.1.1 Absorption by inhalation

Absorption mechanism

The percentage of aluminium absorbed after inhalation is depending on the inhaled compounds (especially, their solubility) and on the granulometry of the aerosol. Insoluble and/or large particles and those not absorbed, are eliminated from the respiratory tract by macrophage entrapment and mainly via mucociliary clearance (and subsequent expectoration or ingestion). Only particles with an aerodynamic diameter under 5 µm can reach alveoli where the soluble aluminium can be absorbed.

Human data

In humans, inhalation of aluminium or aluminium compounds is mostly related to occupational exposure. Many studies conducted in the workplace report exposure to aluminium from different sources (fumes, dust). Exposure of the general population essentially occurs through ingestion, including ingestion of indoor or outdoor dust. A possible source of consumer respiratory exposure is aerosols from antiperspirants or other sprays.

In alveoli, aluminium bioavailability increases with the solubility of the inhaled substances. Studies in workers suggest a mean absorption rate of 1.5-3% of inhaled aluminium (R. A. Yokel et McNamara 2001; ATSDR 2008; SCCS 2020).

In a cross-sectional study, pre- and post-shift aluminium serum and urinary levels were measured in 235 workers from 15 different plants employed in areas with potential exposure to aluminium dust, and 44 unexposed workers. In this study, for exposed workers, median aluminium values were 25 and 100 µg m^{-3} for respirable and total particulates in air, respectively. Significant differences were found between exposed workers and controls for pre-shift serum aluminium only (4.92 µg/L vs 3.60 µg/L respectively). Differences were also noted for both pre-shift and post-shift urinary aluminium (pre-shift: 17.11 µg/L for workers vs 7.39 µg/L for unexposed controls; post-shift: 20.08 µg/L for workers vs 7.67 µg/L for unexposed controls) and urine aluminium/creatinine ratios (pre-shift: 12.06 µg/g vs 6.39 µg/g for workers and controls, respectively; post-shift: 13.74 µg/g vs 5.73 µg/g for workers and controls, respectively) (Gitelman et al. 1995).

In another study, after an 8-hour exposure to 0.3-10.2 mg/m^3 (mean: 2.4 mg/m^3) aluminium in welding fumes, 3 volunteers (not previously exposed) had urinary aluminium concentrations of 15-414 µg/L at the end of shift against less than 3 µg/L before exposure (Sjögren et al. 1985).

Animal data

ATSDR reported studies showing that aluminium is retained in the lung after exposure to inhalable aluminium oxide (Christie, Mackay, and Fisher 1963; Thomson et al. 1986) and aluminium chlorohydrate (Steinhagen, Cavender, and Cockrell 1978; Stone et al. 1979). There were no significant increases in aluminium levels in tissues other than the lungs or serum (Steinhagen, Cavender, and Cockrell 1978; Stone et al. 1979) (ATSDR 2008).

Results of experimental studies on intranasal aluminium-containing compounds, in either solution or in particulate form, on the absorption through the olfactory pathway in rats, show a low olfactory nerve uptake and subsequent neuronal distribution to the cortex of instilled soluble aluminium compounds. Concerning nanoparticular aluminium, studies are very few and the results are controversial, with one study showing some transfer (aluminium nanoparticles) (Kwon et al. 2013) while another did not (nanometric aluminium oxides) (Chalansonnet et al. 2018).

4.1.1.2 Absorption by oral route

Absorption mechanism

Aluminium is poorly absorbed following oral intake. This oral absorption depends on several factors, including the concerned aluminium species, its solubility, and the presence of complexing ligands or competing ions (Affourtit, Bakker, et Pronk 2020).The bioavailability appears to be generally associated with water solubility; however, there is insufficient data to directly extrapolate from solubility in water to bioavailability (ATSDR 2008). Aluminium's gastrointestinal absorption from aluminium compounds highly depends on its ionic availability in the gut content, which is mainly linked to the prevailing pH, the present complexing ligands which the metal may form absorbable aluminium species with and also the ingested aluminium compound's chemical form. Acid digestion in the stomach is thought to degrade most of the ingested aluminium compounds to generate "free" and soluble Al^{3+}, some of which might be complexed with mono-, di- and tricarboxylic acids like citric acid. The increase in pH during the transition from the stomach to the intestines, leads to successive deprotonations and the formation of complexes of aluminium with hydroxide and ultimately, the formation of insoluble aluminium hydroxide at neutral pH.

Consequently, when the pH is neutralised in the duodenum, the aluminium ion is progressively converted to aluminium hydroxide and the majority of it is then expected to precipitate in the intestine, with subsequent excretion in the faeces, leaving only a minor fraction available for absorption (EFSA 2011). The large hydration of the Al^{3+} ion can allow it to cross the intestinal epithelium paracellularly, which is probably the main mechanism of absorption. A neutralisation by complexation (e.g., by the anion of the ingested salt), can facilitate the diffusion of the metal through the intestinal membrane. This absorption is therefore generally perceived as a biphasic process involving fast mucosal uptake, followed by slow transport of aluminium into the blood.

On the other hand, a transferrin/vitamin D-dependent active transport is also involved where aluminium shares absorption pathways with other mineral cations (e.g. Mg^{2+}, Fe^{2+}); this hypothesis is incompletely elucidated and complicated by the differences in oxidation states between aluminium and iron ions (Berthon 2002; ATSDR 2008).

Human data

Studies conducted on this topic showed that aluminium present in food and drinking water is poorly absorbed through the gastrointestinal tract.

Based on daily aluminium dietary consumption and urinary daily excretion, the oral global bioavailability of aluminium from the diet has been estimated to be 0.1-0.3 % (R. A. Yokel et McNamara 2001; ATSDR 2008; EFSA 2008). Absorption of aluminium from drinking water is estimated to be around 0.3 % (R. A. Yokel et McNamara 2001).

Stauber et al., 1999 estimated, using 29 healthy volunteers, the relative bioavailability of aluminium species naturally present in food and in aluminium-treated drinking water (ATW) (aluminium compound not specified) (ATW ;140±9 µg/L of Al). Volunteers drank ATW during two-day periods while on a controlled diet. Volunteers' average total intake of aluminium from food and tea was about 3,000 µg/d while aluminium from ATW was 208 µg/d Al. Only 0.3–0.4% of the aluminium in ATW was absorbed in the digestive tract, a percentage close to that absorbed from food (Stauber et al. 1999). Several studies indicated that simultaneous consumption of citric acid or citrates increases aluminium intestinal absorption. However, though higher than the absorption of most other aluminium compounds, intestinal absorption of aluminium citrate is still low (0.5-5%). In fact, aluminium absorption is probably indirectly facilitated by citrate through binding the Ca^{2+} ions that line the lateral membranes of the intestinal cells and thus, widening the loose junctions between them (Desroches, Daydé, et Berthon 2000). Other carboxylic acids, such as lactic acid or ascorbic acid also enhance aluminium absorption in the digestive tract (ATSDR 2008). Citric acid and other carboxylic acids have the potential to form neutral and more soluble species, making aluminium more available for active-transport pathways (DeVoto et Yokel 1994). Conversely, some

complexing agents (e.g., phosphates or silicates) form insoluble compounds with aluminium, limiting its uptake (DeVoto et Yokel 1994; EFSA 2008; ATSDR 2008).

For oral exposure, there is limited information on the bioavailability of aluminium from sources other than diet and water:

As aluminium in soil is mainly constituted of insoluble species (silicates, oxides, and hydroxides), its bioavailability is expected to be low; this is confirmed by the available studies showing a low oral bioaccessibility[33] of aluminium from soils (median 1.1%; upper limit of the confidence interval of the 95th percentile: 2 %) (Kierulf et al. 2022).
The bioavailability of aluminium hydroxide from antacids is also very low. It was estimated to be 0.004% in volunteers with normal renal function (increasing to 0.2% with concomitant citric acid absorption) (Weberg et Berstad 1986).

Animal data

Several animal studies used [^{26}Al]-aluminium (compound not specified) to estimate aluminium bioavailability from drinking water. In a study by Jouhanneau et al., (compound not specified), when aluminium levels in urine, bone, liver, and brain were considered, an absorption rate of 0.1% was estimated in rats (Jouhanneau et al. 1997). Absorption of soluble salts (citrate, nitrate, sulphate) in the rat was 0.05%-0.2% compared to 0.02%-0.1% for insoluble compounds (oxide, hydroxide, sodium aluminium silicate) (Priest, Skybakmoen, et Jackson 2021). In the same species, oral absorption was greater for aluminium oxide nanoparticles than for aluminium chloride (a soluble salt); absorption of elemental aluminium nanoparticles was lower but still higher than $AlCl_3$ absorption (Krause et al. 2020).

In rats, the bioavailability of aluminium from tea infusion of ^{26}Al citrate injected tea leaves was estimated to be 0.37 % (Robert A. Yokel et Florence 2008); and it was 0.1%-0.3% for aluminium from basic aluminium sodium phosphate (a food additive) in biscuits or cheese (Robert A. Yokel et Florence 2006; 2008). As in humans, numerous studies in animals have shown that the addition of citric acid or lactic acid enhances aluminium absorption through the digestive tract (ATSDR 2008). In a study conducted by Cunat et al., where an *ex vivo* rat gut was used to evaluate the potential of aluminium absorption from different chemical aluminium species, aluminium absorption was positively correlated with the theoretic affinity of aluminium and the anion. The absorption of aluminium after ingestion of organic aluminium compounds was more important than after ingestion of inorganic aluminium compounds, with the following order: aluminium citrate > aluminium tartrate, aluminium gluconate, aluminium lactate > aluminium glutamate, aluminium chloride, aluminium sulphate, aluminium nitrate (Cunat et al. 2000).

4.1.1.3 Absorption by dermal route

Human data

Skin application of personal care and cleaning products appears as one of the main sources of external exposure to aluminium for the general population. In most of the aluminium-containing cosmetics, insoluble species are used. Antiperspirants are a notable exception, as they contain soluble aluminium salts (e.g., aluminium chlorohydrate) and their pH is generally low, increasing the bioaccessibility of aluminium. However, when applied to skin, aluminium compounds form inert complexes with basic components of sweat and skin, limiting the bioaccessibility (SCCS 2014).

Human data on aluminium dermal absorption are limited. Older studies indicate a large range of absorbed fraction values ranging from 0.012% to 10% (SCCS 2014). However, these studies were conducted with a low number of volunteers (1 or 2) or *in vitro* using skin preparations, and their results should be considered with caution.

A study involving 12 volunteers using several exposure scenarios was recently published. It concluded that the aluminium fraction absorbed dermally from antiperspirants ranged from 0.002% to 0.06 % (mean: 0.0094%, standard deviation: 0.0131) (de Ligt et al. 2018). However, SCCS estimated that this study had several methodological flaws (gaps in the mass-balance of ^{26}Al and lack of information on how the missing amounts could be accounted for). SCCS requested a new study from the cosmetic industry, which was finally performed by the Netherlands Organisation for Applied Scientific Research (TNO) and is presented in the SCCS 2020 report (SCCS

[33] Bioaccessibility is defined as the fraction of a compound which can be solubilized in the digestive tract. Bioavalailability is the fraction of this substance which can be distributed in the body. For inorganic compounds bioaccessibility can be considered as a proxy of their bioavailibility.

2020). This recent study with 6 female volunteers showed a mean dermal absorption fraction of 0.00052 % (min-max: 0.00026%-0.00108%) from a topical application of roll-on formulation, containing aluminium chlorohydrate labelled with [^{26}Al]-aluminium (0.75 g antiperspirant per axilla (total of 1.5 g), ~2,500Bq, nominal dose of ^{26}Al of 3730317 pg). The volunteers were biomonitored for 11 days by measuring the presence of ^{26}Al in urine and faeces (de Ligt et al. 2022; SCCS 2020).

At the same time, they biologically monitored their blood, taking regular samples for up to 7 days (0-168h). Combined with the aluminium found in the faeces from the same study (0.0014%), this would yield an overall percentage of bioavailable aluminium of 0.00192%. However, De Ligt et al. (2022) argued against including this additional test material from faeces. SCCS (2020) agreed noting that no paired faecal samples were collected following intravenous (IV) dosing for relative comparison. Thus, the mean dermal absorption value of 0.00052% was regarded by SCCS as an appropriate value to use in risk assessment (SCCS 2020).

Fourteen-day use of aluminium-based antiperspirants did not increase plasma or urine aluminium levels in 21 volunteers (M. Letzel et al. 2020). In this study, shaving habits did not impact the systemic aluminium load.

Animal data

In mice percutaneously exposed to 0.1 or 0.4 μg of aluminium chloride daily for 130 days (i.e., 0.02 - 0.08 μg Al/d), Anane et al., reported a significant increase in aluminium levels in urine, serum and brain. This study shows that aluminium was absorbed through the skin of mice. The concentration of aluminium in serum varied between 317.5 μg/L (SEM ±63.7) and 380.0 μg/L (SEM ±84.6) when mice were exposed to 0.02 and 0.08 μg Al/day respectively (versus 125.0-227.5 μg/L in the control group). It is noteworthy that, in this study, no measures were taken to prevent animals from licking their fur, and thus, ingesting aluminium (Anane et al. 1995).

4.1.2 Distribution

Once in the body, aluminium ion binds to blood proteins and is distributed to all tissues, especially the bones and lung (Ganrot 1986).

Aluminium erythrocyte/plasma partition ratio varies from one publication to another with values between 0.1 and 0.9 (Vesa Riihimäki et Aitio 2012). Around 95% of plasma aluminium is protein-associated, with 80% bound to transferrin at the sites left vacant by iron, 10% bound to albumin, and 5% bound to a low molecular weight protein fraction (Priest 2004). It was demonstrated that aluminium, bound to low molecular weight proteins (LMW–Al), present in spiked human serum from eight healthy volunteers, corresponded to Al-citrate, Al-phosphate and ternary Al-citrate–phosphate complexes (Polak et al. 2001).

It should be emphasized that cellular uptake of aluminium by organs and tissues most likely occurs from the aluminium bound to transferrin. Thus, it is likely that the relative high density of transferrin receptors in different organs influences the distribution of aluminium to organs, with higher aluminium levels present in regions of high transferrin receptor density (ATSDR 2008).

Total aluminium body burden is usually 30-50 mg in healthy adults. In the general population, serum aluminium level is usually 1-3 μg/L and exceptionally above 10 μg/L. Half of the total body burden is in the skeleton and about one fourth in the lung (ATSDR 2008).

Aluminium levels in bone tissue of healthy individuals range from 5 to 10 mg/kg. They are around 20 mg/kg wet weight in lungs of adults; lung aluminium concentration can be higher in workers after prolonged exposure to aluminium-containing respirable particles, especially when the aluminium species in particles have a low solubility (Ganrot 1986; ATSDR 2008). According to ATSDR, an increase in aluminium concentration with age in lung tissue is due to an accumulation of insoluble aluminium compounds that have entered the body via the airways (Ganrot 1986; ATSDR 2008). Ganrot (1986), have also reported data showing aluminium concentration increasing with age in lung, liver, kidney, bone, and brain tissues.

Aluminium concentration levels typically reported in the human brain range from 0.25 to 0.75 mg/kg, with grey matter containing about twice the concentration found in white matter (ATSDR 2008). Increased brain aluminium level is observed in patients with dialysis encephalopathy. Studies in Alzheimer's disease patients inconsistently showed elevated brain aluminium levels (Krewski et al. 2007). It has been suggested that the aluminium flux through the blood-brain barrier results from transferrin receptor-mediated endocytosis of transferrin-bound

aluminium and of transferrin-independent mechanisms involving aluminium citrate. Actually, a study by Yokel et al., showed that the transport of aluminium citrate across the blood–brain barrier is carrier-mediated, involving either an uncharacterized monocarboxylate transporter (MCT) isoform expressed in the brain such as MCT7 or MCT8 and/or one of the many members of the organic anion transporting protein family, some of which are known to be expressed at the blood–brain barrier (Robert A. Yokel et al. 2002).

Aluminium is also present in human skin, lower gastrointestinal tract, parathyroid glands; low aluminium concentrations were measured in most soft tissue organs, other than lungs. Aluminium is also able to cross the placental barrier and accumulate in foetal tissue (ATSDR 2008).

Distribution following intramuscular injection:

Aluminium compounds are commonly used as adjuvants in vaccines making the intramuscular route a pathway of exposure to aluminium for the general population. Once injected in tissues, aluminium-containing adjuvants form an extracellular depot; then, they are slowly solubilised by citrate ions and can enter blood (Affourtit, Bakker, et Pronk 2020).

A study conducted in 12 preterm infants (mean gestational age: 27.1 weeks, mean weight at birth: 1021 g, mean weight at vaccination: 2254 g) did not show a significant change in serum and urine aluminium levels 24 hours after the routine 2-month vaccination, with vaccines containing a total of 1200 µg of aluminium (Movsas et al. 2013). The administered vaccines were Prevnar 13 (containing aluminium phosphate), PedvaxHIB (containing aluminium hydroxyphosphate sulphate) and Pediarix (containing aluminium hydroxide and aluminium phosphate). This shows that serum aluminium does not significantly increase following vaccination.

However, in animals, following intramuscular administration of ^{26}Al hydroxide or ^{26}Al phosphate vaccine adjuvants in rabbits, increased levels of ^{26}Al were found in the kidney, spleen, liver, heart, lymph nodes, and brain (in decreasing order of aluminium concentration) (Flarend et al. 1997). In this study, aluminium from intramuscular deposits of both solutions appeared in blood as early as 1 hour after injection. The area under the curve showed that during the first 28 days after exposure, three times as much aluminium diffused from the aluminium phosphate deposits than from the aluminium hydroxide deposits; the terminal phase of the blood concentration curve was not reached by that time.

In a study conducted in rats, very low aluminium levels were found in brain after administration of aluminium phosphate or aluminium hydroxide adjuvanted vaccines (adjuvant group means 0.14–0.29 µg/g wet weight; control 0.13 ±0.04 µg/g ww), and the authors concluded that the diffusion of aluminium from vaccine deposits to the brain, if there is any, is marginal. In this study, aluminium from both aluminium phosphate and aluminium hydroxide adjuvants, as well as adjuvanted vaccines increased aluminium levels mainly in bone (5-12% of the administered dose). The release from injected muscle was faster for aluminium phosphate than for aluminium hydroxide: 85.5% vs 22.3%, over 80 days. Different rates of absorption were noted, with markedly higher systemic availability from aluminium phosphate than from aluminium hydroxide-adjuvanted vaccines (Weisser et al. 2019).

Other studies conducted in mice have shown that after intramuscular injection, aluminium particles (administered as oxyhydroxide or hydroxide nanodiamants) were slowly translocated to draining lymph nodes, then to blood, spleen, and liver (Khan et al. 2013; Eidi et al. 2015). Crépeaux et al. (2015), in a study conducted in mice, showed that the translocation of aluminium from muscular deposits to lymph nodes and spleen was very slow, with the highest number of particles being observed in these tissues at day 270. In this study, no translocation of aluminium to the brain was observed (Crépeaux et al. 2015).

4.1.3 Metabolism

Aluminium does not undergo metabolism but can be transformed from one species to another. In living organisms, aluminium is thought to exist in four different forms: as Al-ions (free and weakly or more strongly bound in salts), as low-molecular-weight complexes (with organic acids, amino acids, nucleotides, phosphates, and carbohydrates), as physically bound macromolecular complexes (with proteins, polynucleotides, and glycosaminoglycans), and as covalently bound macromolecular complexes (Ganrot 1986; Priest 2004). The free ion, Al^{+3}, is easily bound to many substances and structures; therefore, its fate is determined by its affinity to each of the ligands and their relative amounts as well as metabolism. The low-molecular-weight complexes are often chelates and may be very stable. The macromolecular complexes are expected to be much less active than the LMW-complexes. Aluminium may also

form complexes with macromolecules that are so stable that they are essentially irreversible (Daydé et al. 2003; ATSDR 2008).

4.1.4 Excretion

Aluminium is primarily eliminated in the urine (95%) via the glomerular filtration process, while the unabsorbed dietary aluminium is excreted in the faeces. A minor, secondary route (~ 2%) is excretion *via* bile (Krewski et al. 2007; EFSA 2008). Aluminium has also been detected in breast milk at a typical range of 0.0092 to 0.049 mg/L (ATSDR 2008), as well as saliva, sweat and seminal fluid (Krewski et al. 2007).

Multiple reported values for serum and urine elimination half-lives of aluminium (from hours to years, according to the duration of the period of observation) in humans and animals suggest that there is more than one compartment of aluminium storage, in particular bones and lungs (EFSA 2008; Klotz et al. 2019). Typically, the longer half-life can only be determined with increased duration of sampling, and retention times for aluminium appear to be longer in humans than in rodents. In most individuals, the largest aluminium pool is the skeleton; elimination from bone is very slow with a half-life of several years (Klotz et al. 2019). Slow aluminium elimination coupled with continued exposure may explain the increasing body burden with age (Affourtit, Bakker, et Pronk 2020).

4.1.4.1 Excretion following inhalation

Human data

Urinary excretion is the primary route of elimination of absorbed aluminium following inhalation exposure (Klotz et al. 2019). Elevated levels of aluminium in urine have been detected in aluminium welders and aluminium flake workers (ATSDR 2008). Exposure studies showed that aluminium excretion is biphasic. As reported in ATSDR 2008, the excretion half-life for the first phase ranged from 7.5 to 9 days among workers exposed to welding fumes or aluminium dust (Sjögren et al. 1985; 1988; Pierre et al. 1995). The half-lives for the second phase ranged from 6.8 to 24 weeks (Sjögren et al. 1988; Schaller et al. 2007). The wide range of apparent half-lives reflects differences in the duration of sampling. Furthermore, several investigators (Sjögren et al. 1988; S. Letzel, Schaller, and Angerer 1996) have found a linear relationship between post-shift urinary aluminium levels and levels of aluminium in air around welders during the last or the cumulative preceding shifts (ATSDR 2008).

Animal data

No studies were available in ATSDR (2008) or in the literature search since then.

4.1.4.2 Excretion after oral intake

Human data

An acute exposure of 4 days to 54.3 mg/kg Al, as aluminium carbonate, produced peak concentrations ranging from 4- to 10-fold elevation in base-line urinary levels (<200 µg/day (± SEM) on control days vs 380-580 µg/day (± SEM) after aluminium intake) (Recker et al. 1977). In addition, a 40-day balance study was conducted by Greger and Baier, where eight adult males were fed two levels of aluminium, as aluminium lactate: 5 mg/day for 20 days (control diet) and 125 mg/day for 20 days (test diet). Subjects excreted two- to five-fold more aluminium in their urine when fed the test diet (47 to 212 µg aluminium/day; mean 1.71 mg/kg bw per day) rather than the control diet (24 to 58 µg aluminium/day; mean 0.07 mg/kg bw/day) (Greger et Baier 1983). Patients taking aluminium hydroxide antacids (1-3 g aluminium per day) had a 3-fold increase in urinary aluminium urine and serum levels. For example, e.g.: a subject excreted 0.098 mg of aluminium in urine/day before taking Amphogel 90 (aluminium hydroxide) and then excreted 0.282 mg/d after starting the antiacid. Aluminium concentration in plasma were 36 µg/L and 46 µg/L before and after taking the antacid, respectively (Gorsky et al. 1979).

Animal data

A single oral dose of 11 mg aluminium (administered as aluminium chloride) to healthy Sprague-Dawley rats resulted in a 14-fold increase in urine aluminium levels after 5 days (9.23±2.21 µg/day), as compared to baseline levels (0.40±0.54 µg/day) (Ittel et al. 1987). Rats administered a single dose of one of eight aluminium compounds (each one containing 35 mg aluminium), by gavage, excreted 0.015–2.27% of the initial dose in the urine. In this

experiment, 24-hour urine was collected from rats for 2 days before the gavage and 3 days afterwards (Froment et al. 1989). The range of urinary elimination most likely reflects differences in gastrointestinal absorption (ATSDR 2008).

Faecal aluminium results from unabsorbed aluminium and aluminium excreted via bile (ATSDR 2008). In rats receiving a gavage dose of 6.7–27 mg Al/kg bw (administered as aluminium lactate in a sodium citrate aqueous solution to enhance aluminium absorption from the gastrointestinal tract), 1.3-2.8% of the total dose was excreted in urine and 0.06 to 0.14% of the total dose was excreted in bile within 3 hours. Within 15 minutes, the levels of aluminium in bile were significantly higher than in controls (Sutherland et Greger 1998).

4.1.4.3 Excretion after dermal exposure

Human data

No studies were available in ATSDR (2008) or in literature search since then.

Animal data

No studies were available in ATSDR (2008) or in literature search since then.

4.1.4.4 Excretion after intramuscular injection

In the rabbit study by Flarend et al., the cumulative amount of aluminium eliminated in the urine over 28 days was 6% of the aluminium hydroxide adjuvant dose and 22% of the aluminium phosphate adjuvant dose. Aluminium from both adjuvants was still being excreted at a steady rate on day 28 (Flarend et al. 1997). While this indicates that the body can eliminate the aluminium absorbed from the adjuvants, elimination is slow for the aluminium phosphate adjuvant and even slower for the aluminium hydroxide adjuvant (Affourtit, Bakker, et Pronk 2020).

4.1.4.5 Excretion after intravenous injection

Two studies conducted in healthy human volunteers described aluminium elimination following intravenous injection of [^{26}Al]-aluminium citrate (Priest et al. 1995; Talbot et al. 1995).

In the study by Priest et al. (1995) a single volunteer received 0.7 µg aluminium. After 15 minutes, more than half of ^{26}Al had left the blood and the decline continued afterwards, leaving less than 1 % of the injected dose in the blood after 2 days. This initial rapid decrease resulted from both distribution and excretion. The proportion of the dose excreted, estimated up to 13 days after injection, was 83 % in urine and 1.8 % in faeces. The fraction retained in whole body was 15.2 % at 13 days, and then slowly declined to around 4 % after 1178 days (3 years) (Priest et al. 1995). A re-analysis of these data in a later publication indicated that they could be adequately interpreted by a three-compartment model with half-lives of 1.4, 40, and 1727 days. This volunteer was re-examined 10 years following the injection, and collected data indicated that the current elimination half-life for this volunteer was very prolonged, estimated to be around of 50 years (Priest 2004).

In the study by Talbot et al. (1995) 6 healthy male volunteers received the same injection of [^{26}Al]-aluminium as citrate salt (4 ml containing 84 ng ^{26}Al and 25 mg citrate). Blood concentration decreased by 98 % in one day (mean value of 2% of injection remaining after 1 d); 59 ± 10 % of the aluminium was excreted in the urine within 24 hours, while faecal excretion was negligible during the first 5 days (1 %). Whole-body retention was still 27 ± 7 % at 5 days (Talbot et al. 1995).

Overall, these two studies reported convergent results, evidencing a rapid excretion of most absorbed aluminium in the urine and a fraction that persists a very long time in the body.

4.2 PBPK model

Various kinetic models for aluminium, with different levels of complexity, have been published. In the three models developed below, insoluble aluminium compounds are not considered.

The open compartmental model is represented in **Erreur ! Source du renvoi introuvable.**(Nolte et al. 2001). The parameters of this model are based on 4 studies in rats with oral administration of ^{26}Al-chloride and 3 human studies with IV administration of ^{26}Al-citrate. The essential features of the model are a central compartment, three

compartments for the digestive tract and three peripheral compartments. The central compartment consists of transferrin-bound aluminium and citrate-bound aluminium in plasma and in the interstitial fluid. The peripheral compartments are used for organs such as the liver and spleen, muscles and bones. The liver and spleen receive aluminium from the transferrin-bound aluminium in the plasma, the muscles from the transferrin-bound aluminium in the interstitial fluid and the extracellular bone tissue from the citrate-bound aluminium of the interstitial fluid.

With regards to the gastrointestinal absorption, aluminium uptake is considered to take place in the duodenum and proximal jejunum. Excretion of incorporated aluminium occurs from the soluble ultrafiltrable phase of the citrate-bound aluminium in the plasma via the kidneys. Measured values of ^{26}Al in serum and urine were used to determine absorption, speciation, distribution, retention, and excretion of aluminium in healthy volunteers and in patients with chronic renal failure following administration of a single oral or IV dose of ^{26}Al (Steinhausen et al. 2004).

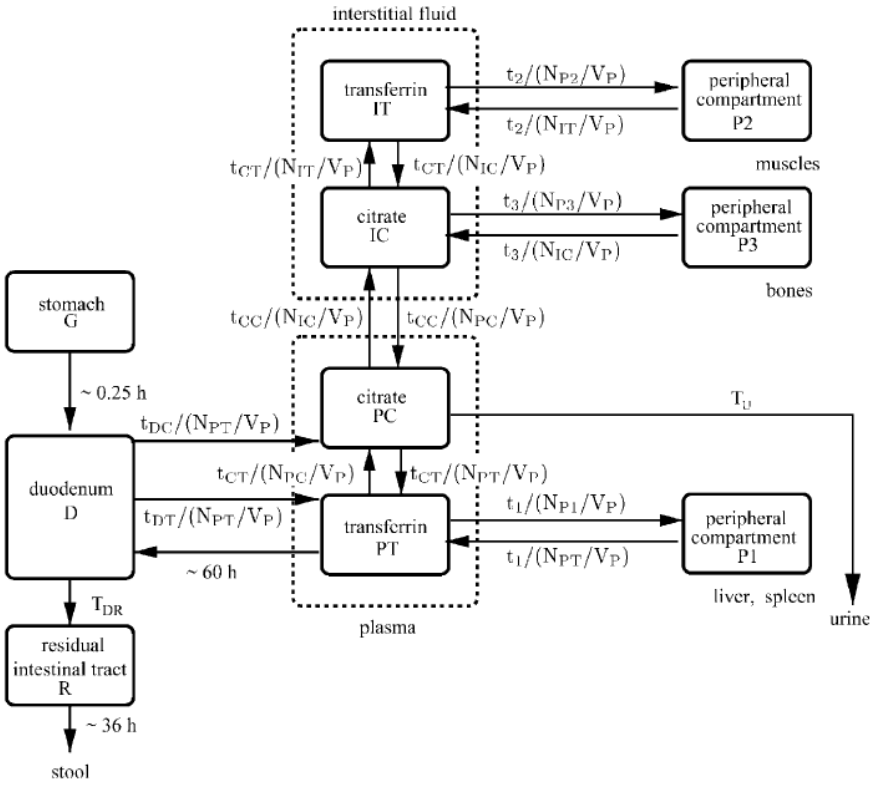

Figure VII.4: Open compartmental model for aluminium biokinetics (Nolte et al. 2001)

The 3-compartment human model is represented in **Erreur ! Source du renvoi introuvable.**. The model tracks the amount of aluminium in each of three compartments (plasma, rapid and slow tissues) as well as excretion in urine. In this model, an additional excretion pathway (combined faecal and dermal) was included to allow modelling of some of the data sets.

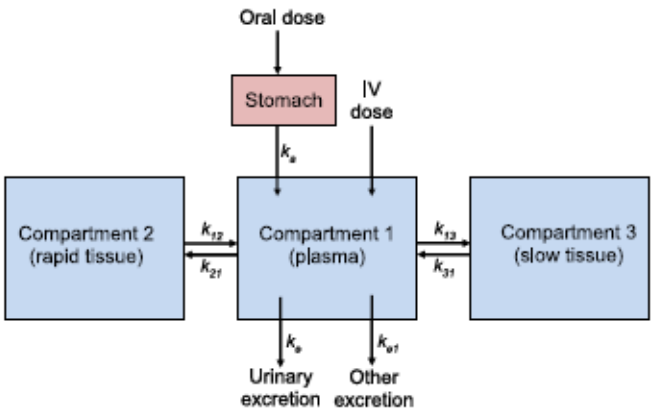

Figure VII.5: Structure of three-compartment aluminium pharmacokinetic model (Poddalgoda et al. 2021)

Hethey et al. (2021) described a physiological-based toxicokinetic model of aluminium citrate or chloride salts in rat and human (**Erreur ! Source du renvoi introuvable.**). This PBK model accounts for aluminium kinetics in plasma, blood, liver, spleen, muscle, bone, brain, kidney, and urine. A 'rest of body' compartment describes the sum of the remaining body spaces (carcass, adipose tissue, lung, and sites not quantified in previously-mentioned tissue homogenates).

The organs are in exchange via a central blood compartment that includes the arterio-venous space as well as the vascular space of the tissues. In the figure below, mass transfers are indicated by black arrows. Different aluminium species are considered in the blood compartment: iv-administered citrate, and chloride salts as well as a 'mixed' state, where all aluminium species, including transferrin bound Al, are assumed to be in quasi-steady state. Routes of administration are indicated by red arrows, including intravenous and oral administration. In this model, they assumed the amount of aluminium in erythrocytes to be negligible compared to the amount in plasma.

The detailed model structure with three aluminium states in blood was designed to account for potential differences in renal elimination depending on the route and aluminium salt (citrate or chloride) administered.

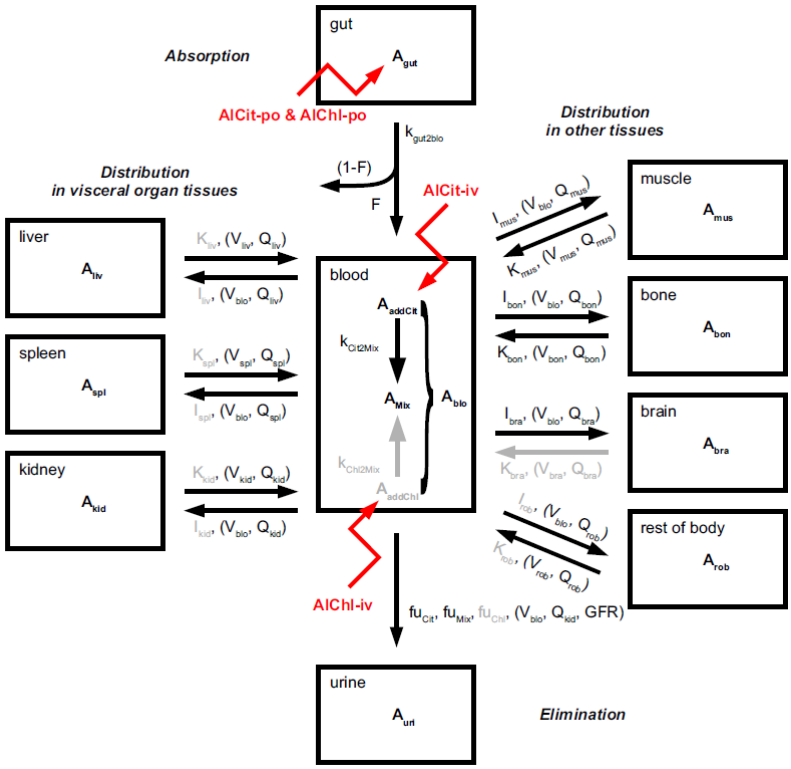

Figure VII.6: PBK model structure of aluminium (Hethey et al. 2021)

4.3 Overview ADE (absorption, distribution, excretion)

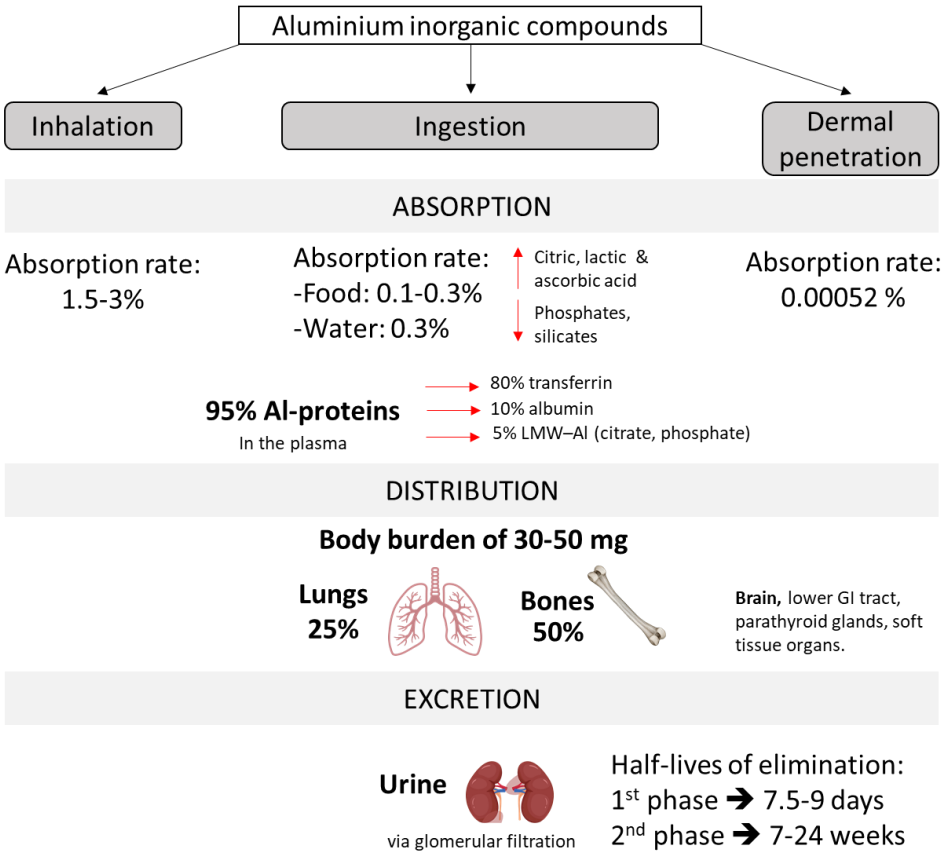

Figure VII.7: Absorption, distribution and excretion of aluminium inorganic compounds in human

The aluminium load in the human body, from daily absorption through the digestive, pulmonary, and even dermal routes, is distributed mainly to the bones (50%) and the lungs (25%); aluminium is also found in small amounts, in the brain, the lower gastrointestinal tract and soft tissue organs other than the lungs. The transport protein of aluminium (as Al^{+3}) is transferrin, and the main route of excretion is the urinary route.

4.4 Acute and subacute toxicity

4.4.1 Human data

No reliable studies have been identified regarding the effects of acute inhalation or ingestion of aluminium or its compounds in humans.

Several cases of aluminium related encephalopathy were reported in patients who underwent otoneurosurgery with bone reconstruction using an aluminium-containing cement (Hantson et al. 1995; Lévêque et al. 1996; Reusche et al. 2001).

Cases of acute encephalopathy with high aluminium plasma levels are also reported, after post-surgical bladder alum irrigation. However, in most of these cases, aluminium was probably not the only or the main cause of the neurological symptoms, as severe hydroelectrolytic disturbances were obviously or probably associated (Phelps et al. 1999).

4.4.2 Animal data

Oral exposure

ATSDR (2008) reported several acute toxicity studies in animal following **oral exposure** to aluminium or its compounds.

- **Mice**

In mice (Swiss-Webster), LD_{50} (median lethal dose) values were 286, 222 and 164 mg Al/kg bw for aluminium nitrate, aluminium chloride and aluminium bromide, respectively (Llobet et al. 1987). In another mice strain (Dobra Voda), LD_{50} values were higher, with 770 mg Al/kg bw per day for aluminium chloride and 980 mg Al/kg bw per day for aluminium sulphate (Ondreicka, Ginter, and Kortus 1966).

- **Rats**

The LD_{50} values in rats were 261 mg Al/kg bw for aluminium nitrate, 370 mg Al/kg bw for aluminium chloride, 162 mg Al/kg bw for aluminium bromide and >730 mg Al/kg bw for aluminium sulphate (Llobet et al. 1987).

- **Rabbits**

5/5 female New Zeeland rabbits died from a single 540 mg Al/kg bw dose of aluminium lactate administered by gavage (R. A. Yokel and McNamara 1985).

Inhalation exposure

Following acute **inhalation,** ATSDR (2008) found no studies assessing mortality of aluminium or its compounds in animals and confirmed that no lethality has been reported in animal studies following this type of exposure. However, some respiratory effects following acute and subacute inhalation of aluminium compounds have been reported.

- **Mice**

Mice (n=11) exposed in a whole-body chamber, for one hour, to a suspension of 8 mg/m^3 of aluminium dust had a significantly higher fraction of alveolar collapse (69.7±1.2%) and influx of poly-morphonuclear cells in lung parenchyma (27.5±1.1%) than the control group (n=11) exposed to saline (Mazzoli-Rocha et al. 2010).

- **Rats**

In male rats (Fischer-344, 12-18 per dose) inhaling 0, 10, 50 100, 200 or 1000 mg/m^3 aluminium flakes (median diameter: 1.58 µm) for (5x) 4 hours, multifocal microgranuloma in the lungs and hilar lymph nodes were detected at the 2 highest concentrations starting 200 mg Al/m^3. An increase of lactate dehydrogenase, glucose-6-phosphate dehydrogenase, and alkaline phosphatase activities in lavage fluid was observed at 50 mg Al/m^3. No respiratory effects were observed at the concentration of 10 mg Al/m^3 (NOAEL: No Observed Adverse Effect Level) (Thomson et al. 1986).

- **Hamsters**

In a series of studies conducted by Drew et al., on hamsters (Golden Syrian), a 13% increase in lung weight was observed after inhalation of aluminium chlorohydrate for 3 days (4 h/d) from the dose of 7 mg Al/m^3 (LOAEL: Lowest Observed Adverse Effect Level), and no significant increase of the lung weight was observed at 3 mg Al/m^3 (NOAEL). An alveolar wall thickening, and an increased number of macrophages were observed after a 3-day exposure (4 or 6 hr/d) to 31 or 33 mg Al/m^3. In this experiment, a progressive decrease in the severity of the pulmonary effects was observed after the end of exposure (Drew et al. 1974).

- **Rabbits**

Similar effects were observed in New Zealand rabbits. In this species, a thickening of the alveolar wall, an increase in the number of macrophages and a 65% increase in lung weight were observed after inhalation of aluminium chlorohydrate for 5 days (4hr/d) at a LOAEL of 43 mg Al/m^3 (Drew et al. 1974).

4.5 Irritation

As indicated in section 3.3, anhydrous aluminium chloride is classified as skin corrosive (category 1B) under the harmonised CLP classification. In addition, the ECHA website[34] reports several CLP notifications received and information from REACH registration dossiers concerning the skin and/ or ocular irritation effect of other aluminium compounds including, aluminium citrate, aluminium hydroxide, aluminium lactate, aluminium nitrate, aluminium phosphate, aluminium silicate, aluminium sodium dioxide and aluminium sulphate.

In fact, it is predictable that, in contact with a humid environment, partially hydrolysed aluminium salts may cause irritation to the skin and mucous membranes following the release of the corresponding acid.

4.5.1 Human data

SCCS mentioned some studies reporting skin irritation following the application of a high dose of aluminium hexahydrate chloride (ACH) in ethanol (20% ACH) in a formulation for the treatment of axillary / palmar hyperhidrosis (excessive sweating) (Ellis and Scurr 1979; Goh 1990; Reisfeld and Berliner 2008) and following the use of aluminium-containing crystal deodorant (Gallego, Lewis, and Crutchfield 1999). SCSS also explained that, although associations have been reported between axillary irritation and aluminium chloride in antiperspirants for the treatment of hyperhidrosis, the history of use of this compound in antiperspirants shows that this effect is not very common (SCCS 2023).

Furthermore, the review by Krewski et al. reported cases of contact dermatitis and irritant dermatitis in workers following exposure to aluminium alloys and aluminium dust. It was also concluded that aluminium inhalation could cause irritation. This is based on several old studies in the work place reporting dry cough and shortness of breath following the exposure to aluminium powder (Krewski et al. 2007).

4.5.2 Animal data

Ocular irritation

Grekhova et al. (1994) reported conjunctivitis and purulent ophthalmitis following the instillation of potassium alum (aluminium potassium sulphate) and ammonium alum into rabbit's eyes (Krewski et al. 2007).

Dermal irritation

SCCS reported a study (Lansdown 1973) in which epidermal lesions, hyperkeratosis, acanthosis (thickening of the epidermis) and micro-abscesses were observed in mice, rabbits and pigs after 5 days of dermal application (once per day) of aluminium chloride or nitrate (10% [w/v]), but not with aluminium acetate, hydroxide or hydrochloride compounds (SCCS 2023).

4.6 Sensitisation

4.6.1 Human data

Aluminium is extensively used in several industries but only a small number of cases of skin sensitization were reported: one report from an aircraft worker and one from a hospital attendant. ATSDR reported a case study described by De Vuyst et al. (1987) where T-helper lymphocyte alveolitis and blastic transformation of peripheral blood lymphocytes in the presence of soluble aluminium compounds *in vitro* were observed in an individual exposed to metallic aluminium and aluminium dust with sarcoid-like epithelioid granulomas (ATSDR 2008).

ATSDR (2008) also reported hypersensitivity to aluminium chloride in patch testing in some children and one adult who had previously received injections of vaccines or allergens in aluminium-based vehicles (Böhler-Sommeregger and Lindemayr 1986; Veien et al. 1986). Yokel and Sjögren (2022) also reported subcutaneous and itching nodules, following the injection of aluminium-containing vaccines. A case of contact urticaria due to aluminium-containing coins was also described (Forte et al. (2008) from (Yokel et Sjögren 2022).

[34] https://echa.europa.eu/information-on-chemicals (accessed on February 2024)

SCCS specified that there is no sufficient data in humans suggesting that aluminium compounds used in antiperspirants cause allergies and given their widespread use, this effect, if it exists, seems to be rather rare (SCCS 2023).

Furthermore, a delayed hypersensitivity granulomatous reaction was reported in a woman undergoing a blepharopigmentation with a pigment containing aluminium silicate (Schwarze et al. (2000) from (Yokel et Sjögren 2022),

4.6.2 Animal data

SCCS reported a study conducted by Basketter et al. (1999) where doses up to 25% of aluminium chloride were tested in a murine local lymph node assay (LLNA), which gave no indications of a skin sensitisation potential. A guinea pig maximisation test with aluminium chlorohydrate dosed at 25 %, was also negative (SCCS 2020).

SCCS confirmed that animal data do not indicate a skin sensitisation effect of the aluminium compounds used in antiperspirants (SCCS 2023).

4.7 Subchronic toxicity

No **human** reliable studies were identified on health effects following subchronic exposure to aluminium compounds.

Among the studies identified in **animals**, seven involved administering aluminium chloride to rats via drinking water at dose levels ranging from 0 to 8.3 mg/kg bw per day (Martinez et al. 2017a, 2017b, 2017c, 2018) or by gavage at dose levels of 0, 8.3 or 32 mg/kg bw per day (Fernandes et al. 2020; Souza-Monteiro et al. 2021; Bittencourt et al. 2022) or 0-100 mg/kg bw per day (Martinez et al. 2017a, 2017b, 2017c, 2018). These studies investigated effects on bone mineralization (Souza-Monteiro, 2021), cardiotoxicity (Martinez et al., 2017a), male reproductive toxicity (Martinez et al., 2017b), and neurotoxicity (Martinez et al. 2017c, 2018; Fernandes et al. 2020; Bittencourt et al. 2022).

All the studies referred to human dietary levels of exposure to calculate the doses to be used in rodent studies, but the human values used for calculation were not clearly justified and did not refer to benchmark values calculated by regulatory agencies. In addition, the doses received by the animals were not confirmed by analysis of aluminium chloride in drinking water nor supported by drinking water consumption measurement. A high level of uncertainty regarding the doses received by the animals led the experts not to consider these studies for the hazard assessment of aluminium chloride.

4.7.1 Neurotoxicity

4.7.1.1 Human data

No reliable human studies were identified on neurotoxic effects following subchronic exposure to aluminium compounds.

4.7.1.2 Animal data

Several animal studies (mice and rats) have been identified on neurotoxicity after subchronic exposure to aluminium compounds. These studies are described below and summarised in Table VII.51.

- **Mice**

Swiss-Webster female mice (dams) exposed to aluminium lactate from Gestational day (Gd) 1 to Lactation day (Ld) 21 through diet did not show signs of toxicity in the neurobehavioral test battery (fore and hindlimb grip strength, temperature sensitivity, negative geotaxis, air puff startle reflex, auditory startle reflex, foot splay) when they were exposed to 25 (control), 500 or 1000 µg Al/g diet (Donald et al. 1989). The NOAEL was 1000 µg/g diet, equivalent to 330 mg Al/kg bw per day, referring to the highest tested dose.

Female Swiss-Webster mice (8-12 weeks of age) were exposed to aluminium lactate for 6 weeks in the diet at doses of 25 (control), 500 or 1000 µg Al/g diet. In the high dose group, there was a significant reduction in total activity counts, as well counts of vertical movement and a significantly lower percent of activity in the highest activity level

category (Golub et al. 1989). The NOAEL was 500 µg/g diet, that is 62 mg Al/kg bw per day and the LOAEL 1000 µg/g diet, that is 130 mg Al/kg bw per day.

Swiss-Webster female mice (dams) exposed to aluminium lactate during in the diet at doses of 25 (control) or 1000 µg Al/g diet during gestation or lactation or, both, did not show effects of aluminium exposure in the neurobehavioral test battery performed at time of weaning (Golub, Keen, et Gershwin 1992a). The NOAEL was 1000 µg/g diet, that is 250 mg Al/kg bw per day, referring to the highest tested dose.

Golub et al. administered aluminium lactate (25 (control) or 1000 mg Al/kg diet) to female Swiss-Webster (3-4 weeks old) mice through diet for 13 weeks. In the high dose group, a decreased forelimb and hindlimb grip strengths and startle response, a decreased in total activity counts, horizontal activity counts and in the percentage of intervals with high activity counts were reported (Golub et al. 1992b). The LOAEL for this study was 1000 mg/kg diet, that is 195 mg Al/kg bw per day.

Aluminium chloride was also given to 42-day-old female Swiss Webster mice through the diet for 5-7 weeks at dose of 3 (control) or 1000 mg Al/kg diet. A decrease in forelimb and hind limb grip strength was reported at the high dose (Oteiza et al. 1993). The LOAEL for this study was 1000 mg/kg diet, that is 195 mg Al/kg bw per day.

Aluminium ammonium sulphate was administered in drinking water to C57BL/6J female mice at 0 or 0.075 g/L from 4 to 9 weeks of age (20 per group) or to both male and female mice at 0 or 1 g/L from Gd 11 until 9 weeks of age (20 per group). Behavioural effects were observed in mice from the low dose group (increase in total arm entries in the elevated plus maze test, initial decrease followed by increase in immobility in the forced swim test, decreased freezing in the fear conditioning test 1 month after the conditioning session compared with controls). However, considering that only one dose was tested and that these effects were not observed in the high-dose group, it appears that the observations were probable false positives, even if the exposure protocols are not identical at the lower and the higher doses. Indeed, these behavioural differences did not reach a statistically significant level after correction for multiple testing. In the high dose experiment, increased social contacts, impaired reference memory performance, decreased prepulse inhibition and reduced correct responses in the working memory task were observed. The differences in any of the behavioural measures, in this second experiment did not reach the significance level after correction for multiple testing (Shoji et al. 2018).

- **Rats**

When aluminium lactate was administered through gavage to Wistar rats, from PND 5 to PND 14, at doses of 0, 100, 200 or 300 mg Al/kg bw per day, no effects were observed regarding the grasping reflex, the negative geotaxis test was significantly reduced at 200 and 300 mg Al/kg bw per day compared to the control group and significant differences were observed in the suspension test (reduced time of suspension) and the locomotor coordination test (increased time to place all 4 feet on the platform) between the control group in the 300 mg Al/kg bw per day dose group (V. Bernuzzi, Desor, et Lehr 1989b). The LOAEL was 200 mg Al/kg bw per day and the NOAEL was 100 mg Al/kg bw per day.

Furthermore, increased brain aluminium levels with decreased choline acetyltransferase activity and general activity were reported at a LOAEL of 200 mg Al/kg bw per day in Wistar rats also orally exposed to aluminium lactate through gavage from Post Natal Day (PND) 5 To PND 14 at doses of 0, 100, or 200 mg Al/kg bw per day (Cherroret et al. 1992). The NOAEL was 100 mg Al/kg bw/day.

Male rats (16 days old and 18 months old- strain not specified) were exposed to aluminium nitrate in drinking water at doses of 0 or 100 mg Al/kg bw per day (citric acid was added to water) for 100 days. No significant effects of aluminium exposure on behaviour (motor activity in an open-field apparatus and learning using passive avoidance test) could be detected between groups. The total number of synapses in the left CA1 fields of hippocampal formation was decreased in the aged aluminium-exposed group compared to the young aluminium-exposed group (M. Teresa Colomina et al. 2002). The NOAEL was 100 mg Al/kg bw per day, the highest tested dose.

In the study by Mameli et al., Wistar rats (n= 270) at 3, 10- and 24-months of age, were exposed to aluminium chloride drinking water (0.5, 1, 2 g/l in drinking solution providing 11.1, 21.5, 43.1 mg Al/kg bw per day) for 90 days. The study also included control groups (Mameli et al. 2006). Rats were tested at 30, 60 and 90 days of exposure. Considering the amount of aluminium present in the diet, they were approximatively exposed to 20, 30 or 52 mg Al/kg bw per day (EFSA 2008). Results showed significant impairment of post-rotatory nystagmus (PRN)

parameters (delayed onset latency of PRN, decrease in jerk frequency and jerk amplitude), regardless of animal age, in rats exposed to 52 mg Al/kg bw per day (LOAEL). NOAEL was considered to be 30 mg Al/kg bw per day.

Twelve months old male Wistar rats (12-13 per group) received 0, 2 or 20 ppm of aluminium in their drinking water, as aluminium chloride, which resulted in an exposure of total aluminium to 0.4, 0.5 and 1.7 mg Al/ kg bw per day, respectively, considering aluminium from the feed. From 6 months of age, rats were trained to perform a rewarded continuous alternation T-maze task. Performance scores on T-maze task were analysed from 16 months of age onwards. Among the rats who survived to at least 28 months, 0/10 in the low-dose group, 2/10 in the mid-dose group and 7/10 in the high-dose group, had significantly lower scores in old age (> 28 months old) compared to middle age (12-24 months old). Rats with impaired performance had significantly higher aluminium serum levels and a greater percentage of aluminium-loaded pyramidal cells in their entorhinal cortex, compared to rats with intact T-maze performance. Furthermore, functionally impaired rats had aberrant behaviours including inability to focus on their task, perseverative activity, and incontinence while in the T-maze. LOAEL for impaired cognitive function was 0.5 mg Al/kg bw per day (Walton 2009). JECFA considered this study difficult to interpret because, among other considerations, rats had an unusual feeding regimen (JECFA 2012).

In another study Wistar rats also received aluminium chloride in their drinking water at a concentration of 0 or 3 g/L for 4 months in adulthood starting 3 months of age. There was a significant increase of glial fibrillary acidic protein (GFA-P)-immunoreactive astrocytes in brains of aluminium treated rats who also had a significantly reduced locomotor activity compared to controls (Erazi et al. 2010). The LOAEL was 3g/L in water, that is 55 mg Al/kg bw per day.
In another study, Wistar rats (15 per group) were administered aluminium chloride at a concentration of 0, 0.18, 0.72 or 3.6 g $AlCl_3$/l in drinking water for 6, 12 and 18 months. A significant increase in aluminium chloride concentration in plasma and brain was observed in a dose dependent and time dependent manner. Exposure to aluminium chloride caused a significant decrease in body weight (highest dose, month 18) and brain weight (mid and highest doses at 18 months); a significant correlation was confirmed between body and brain weight during 12 and 18 months. Aluminium chloride produced, at all doses, lesions of sub-granular and granular layers (significant at month 18) (Hichem et al. 2014). The LOAEL was 0,18 g/L aluminium chloride in water, that is 2 mg Al/kg bw per day.

In a study by Cao et al., aluminium chloride was administered, via gavage, to Sprague-Dawley rats (30 per group) for 90 days at doses of 0, 50, 150 or 450 mg $AlCl_3$/kg per day. Increased mRNA levels of IL-1b (Interleukin-1 beta), IL-6 (Interleukin-6), TNF-a (Tumour Necrosis Factor-alpha) and MCH II (Major Histocompatibility Complex Class II), decreased mRNA levels of CX3CL1 (Fractalkine) and BDNF (Brain-Derived Neurotrophic Factor), as well as reduced dendritic spine density and impaired learning and memory were reported following aluminium chloride exposure in a dose dependent manner but were significant from the mid dose exposure (Cao et al. 2016). The NOAEL was 50 mg $AlCl_3$/kg per day, that is 10 mg Al/kg bw per day and the LOAEL was 150 mg $AlCl_3$/kg per day, that is 30 mg Al/kg bw per day.

Wistar rats (30 per group) were orally exposed to aluminium chloride, from birth until 3.5 months of age, with early exposure via maternal milk for 3 weeks, then after weaning, continued exposure via drinking of water, from post-natal 3 weeks to 14 weeks, at doses of 0.015, 0.030 or 0.045 mol/L (equivalent to 2, 4, 6 g/L). Following aluminium chloride exposure, aluminium concentration in blood increased in a dose-dependent manner, morphology of the hippocampus and neuronal ultrastructure were aberrant, the escape latency and distance travelled became longer in the Morris water maze test and intracellular Ca^{2+} and cAMP levels in hippocampus cells declined significantly (Yan et al. 2017). The LOAEL was 2 g/L aluminium chloride equivalent to 36 mg Al/kg bw per day.

Neurotoxicity of aluminium was also assessed in 3-month-old male Wistar rats (6 per group) administered aluminium chloride over 60 days at doses of 0, 1.5 or 8.3 mg Al/kg bw per day through drinking water or over 42 days at doses of 0 or 100 mg Al/kg bw per day by gavage. Impaired results on object recognition memory were reported in all treated groups. Reactive oxygen species (from low dose) and lipid peroxidation (from 8.3 mg/kg bw) were increased and, AChE activity as well as hippocampal antioxidant capacity were decreased in all treated groups (Caroline S. Martinez et al. 2017c). Martinez et al. have also conducted another study were Wistar rats (8 per group) were administered 0 or 8.3 mg Al/kg bw (dose similar to human dietary levels) through drinking water for 60 days or 0 or 100 mg Al/kg bw through gavage for 42 days. In all treated groups, the development of

mechanical allodynia (pain from normally non-painful stimuli), catalepsy (immobility or rigidity), increased inflammation in the sciatic nerve and systemic oxidative stress were reported. Aluminium was also retained in the sciatic nerve (Caroline Silveira Martinez et al. 2018). The LOAEL was 1.5 mg Al/kg bw per day for the first study and 8.3 mg Al/kg bw per day for the second one.

Wistar rats (10 per group) received aluminium chloride solutions by gavage at doses of 0, 8.3 or 32 mg/kg bw per day for 60 days. At the high dose group, aluminium level increased significantly in hippocampal parenchyma. Aluminium at both concentrations affected long-term memory and induced oxidative stress in prefrontal cortex and hippocampus but did not affect short-term memory (Fernandes et al. 2020). The LOAEL was 8.3 mg mg/kg bw per day of aluminium chloride, that is 1.7 mg Al/kg bw per day.

In another study, male Wistar rats (12 per group) received through gavage either 0 or 8.3 mg/kg bw per day of aluminium chloride for 60 days. Aluminium exposed rats had significantly increased learning deficiency and spatial memory impairment, deregulation of proteins expression, including the ones involved in the regulation of the cytoskeleton, cellular metabolism, mitochondrial activity, redox regulation, nervous system regulation, and synaptic signalling, as well as reduced hippocampal cell body density in CA1, CA3, and hilus (Bittencourt et al. 2022). The LOAEL was 8.3 mg/kg bw per day of aluminium chloride, that is1.7 mg Al/kg bw per day.

In another study by Massand et al., male albino Wistar rats (6 per group) were orally exposed to aluminium chloride via gavage at doses of 0, 100 or 300 mg/kg bw per day for 30 days. Results showed that rats exposed to aluminium chloride had significant damage in all hippocampal regions compared to the control group. At 300 mg/kg bw per day, marked neuronal damage in CA1 and CA3 regions were reported, in comparison with the group exposed to the lower aluminium dose (Massand et al. 2022). The LOAEL was 100 mg/kg bw per day aluminium chloride, that is 20 mg Al/kg bw per day.

Table VII.51: Animal studies on aluminium neurotoxicity

Strain	Duration and route of exposure	Dose	Aluminium Form	Endpoint	NOAEL	LOAEL	Reference
Mice							
Swiss-Webster (n= 16 per group)	Gd1 to Ld 21 In the diet	25 (control), 500 or 1000 µg Al/g diet	Aluminium lactate	Neurobehavioral test battery No effect was observed at the highest dose	330 mg Al/kg bw per day		Donald et al. (1989)
Swiss-Webster (n= 5 per group)	6 weeks In the diet	25 (control), 500 or 1000 µg Al/g diet	Aluminium lactate	Reduced total activity counts, counts of vertical movement and a lower percent of activity in the highest activity level category	62 mg Al/kg bw per day	130 mg Al/kg bw per day	Golub et al. (1989)
Swiss-Webster (Control: n = 14; Al: n = 9)	Gd1-Gd21 or Ld1-Ld21 or Gd1-Ld21 In the diet	25 (control) or 1000 µg Al/g diet	Aluminium lactate	Neurobehavioral test battery No effect was observed at the highest dose	250 mg Al/kg bw per day		Golub et al. (1992a)
Swiss-Webster (n= 12 per group)	13 weeks In the diet	25 (control) or 1000 mg Al/kg diet	Aluminium lactate	Decreased forelimb and hindlimb grip strengths and startle response, decreased total activity counts		195 mg Al/kg bw per day	Golub et al. (1992b)
Swiss Webster (n= 10 per group)	5-7 weeks In the diet	3 (control) or 1000 mg Al/kg diet	Aluminium chloride	Decrease in forelimb and hind limb grip strength		195 mg Al/kg bw per day	Oteiza et al. (1993)

Strain	Duration and route of exposure	Dose	Aluminium Form	Endpoint	NOAEL	LOAEL	Reference
Rats							
Wistar (n= 25-25-29-38 respectively)	PND 5 to PND 14 Gavage	0, 100, 200 or 300 mg Al/kg bw per day	Aluminium lactate	Reduced negative geotaxis test	100 mg Al/kg bw per day	200 mg Al/kg bw per day	Bernuzzi, Desor & Lehr (1989b)
Wistar (n= 4 per group)	PND 5 to PND 14 Gavage	0, 100, or 200 mg Al/kg bw per day	Aluminium lactate	Increased brain aluminium levels with decreased choline acetyltransferase activity and general activity	100 mg Al/kg bw per day	200 mg Al/kg bw per day	Cherroret et al., 1992
(NS) (n= 16 per group)	100 days In water	0 or 100 mg Al/kg bw per day (+citric acid added)	Aluminium nitrate	Motor activity in an open-field apparatus and learning using passive avoidance test No effect was observed	100 mg Al/kg bw per day		Colomina et al., 2002
Wistar (n= 20-30 per group)	90 days In water	11.1, 21.5, 43.1 mg Al/kg bw per day	Aluminium chloride	Impairment of post-rotatory nystagmus parameters	30 mg Al/kg bw per day	52 mg Al/kg bw per day	Mameli et al., 2006
Wistar (n= 5 per group)	4 months In water	0 or 3 g/l	Aluminium chloride	Reduced locomotor activity		55 mg Al/kg bw per day	Erazi et al., 2010
Wistar (n =15 per group)	6, 8 and 12 months In water	0.18, 0.72 or 3.6 g /l	Aluminium chloride	Lesions of sub-granular and granular layers in hippocampus		2 mg Al/kg bw per day	Hichem et al.(2014)
Sprague Dawley (n= 30 per group)	90 days Gavage	0, 50, 150 or 450 mg/kg per day	Aluminium chloride	Impaired learning and memory	10 mg Al/kg bw per day	30 mg Al/kg bw per day	Cao et al. (2016)
Wistar (n=30 per group)	Birth to 14 weeks of age Lactation then in water	2, 4, 6 g/l	Aluminium chloride	Morphological injury of the hippocampus and neuronal ultrastructure longer escape latency and distance travelled the Morris water maze test		36 mg Al/kg bw per day	Yan et al. (2017)

Strain	Duration and route of exposure	Dose	Aluminium Form	Endpoint	NOAEL	LOAEL	Reference
Wistar (n= 6 per group)	60 days in water	0, 1.5 or 8.3 mg Al/kg bw per day	Aluminium chloride	Impaired results on object recognition memory		1.5 mg Al/kg bw per day	Martinez et al. (2017c)
	42 days by gavage	0 or 100 mg Al/kg bw per day					
Wistar (n= 8 per group)	60 days in water	0 or 8.3 mg Al/kg bw per day	Aluminium chloride	Development of mechanical allodynia, catalepsy		8.3 mg Al/kg bw per day	Martinez et al. (2018)
	42 days by gavage	0 or 100 mg Al/kg bw per day					
Wistar (n=10 per group)	60 days Gavage	0, 8.3 or 32 mg/kg	Aluminium chloride	Impairment of long-term memory		1.7 mg Al/kg bw per day	Fernandes et al. (2020)
Wistar (n= 12 per group)	60 days Gavage	0 or 8.3 mg/kg bw/day	Aluminium chloride	Increased learning deficiency and spatial memory and, reduced hippocampal cell body density		1.7 mg Al/kg bw per day	Bittencourt et al. (2022)
albino Wistar (n= 6 per group)	Gavage 30 days	0, 100 or 300 mg/kg bw per day	Aluminium chloride	Damage in hippocampus regions		20 mg Al/kg bw per day	Massand et al. (2022)

4.7.2 Respiratory toxicity

4.7.2.1 Human data

No human studies on respiratory toxicity were identified following subchronic exposure to aluminium compounds.

4.7.2.2 Animal data

Some animal studies (rats, hamsters and guinea pigs) demonstrating respiratory toxicity following a subchronic exposure to aluminium compounds have been identified. These studies are described below and summarised in Table VII.52.

- **Rats**

The inhalation of aluminium chlorohydrate by rats (Fischer- 344), 0.25, 2.5 or 25 mg/m^3 over six months (5d/wk and 6hr/d) led to an increase in alveolar macrophages and to granulomatous lesions in lung at the medium and high concentrations. The LOAEL of 2.5 mg/m^3 Al chlorohydrate, corresponds to a concentration of 0.65 mg Al/m^3; this increase was dose related. Histological alterations or changes in lung weights did not occur at 0.065 mg Al/m^3 (Steinhagen, Cavender, et Cockrell 1978).

In another study, Fischer-344 rats were exposed to aluminium chlorohydrate for 6 months (5d/wk and 6hr/d), at doses of 0.25, 2.5 or 25 mg/m^3. A statistically significant increase in relative lung weight was observed in both sexes at the higher concentration (LOAEL 25 mg/m^3, corresponding to 6.5 mg/m^3 Al; NOAEL 2.5 mg/m^3, corresponding to 0.65 mg/m^3 Al). In male rats the same effect was observed from the mid concentration (LOAEL = 0.65 mg Al/m^3 and NOAEL=0.065 mg Al/m^3 (Stone et al. 1979).

No organ weight or histological changes were observed in the lungs of Sprague-Dawley rats exposed to 70 mg/kg bw per day Al as aluminium chloride in drinking water for 30, 60, or 90 days (base dietary aluminium was not reported) (Dixon, Sherins, et Lee 1979).

In the study by Gomez et al., Sprague-Dawley rats exposed to aluminium nitrate through water over one month at doses of 0, 375, 750 or 1500 mg/kg bw per day had also no organ weight or histological changes of the lungs (Gómez et al. 1986). The NOAEL was of 1500 mg/ kg bw per day of aluminium nitrate, equivalent to 190 mg Al/kg bw per day (the highest tested dose).

- **Hamsters**

In Hamsters (Golden Syrian) inhaling 10 mg Al/m^3 as aluminium chlorohydrate over 5 or 6 weeks (5d/wk and 6hr/d), granulomatous nodules and thickening of alveolar walls due to infiltration of macrophages and heterophils were observed (Drew et al. 1974).

- **Guinea pigs**

The inhalation of aluminium chlorohydrate by guinea pigs (Hartley) over six months (5d/wk and 6hr/d) led to an increase in alveolar macrophages and to granulomatous lesions in lung at a dose of 0.65 mg Al/m^3 (LOAEL), this increase was dose related. Histological alterations or changes in lung weights did not occur at 0.065 mg Al/m^3 (Steinhagen, Cavender, et Cockrell 1978).

Guinea pigs were exposed to aluminium chlorohydrate for 6 months (5d/wk and 6hr/d), at concentrations of 0.25, 2.5 or 25 mg/m^3. A statistically significant increase in relative lung weight was observed in both sexes at the higher concentration (LOAEL 25 mg/m^3, corresponding to 6.5 mg/m^3 Al; NOAEL 2.5 mg/m^3, corresponding to 0.65 mg/m^3 Al) (Stone et al. 1979).

Table VII.52: Animal studies on aluminium sub-chronic exposure respiratory toxicity

Strain	Aluminium compound	Duration and exposure route	Concentra-tion	Endpoint	NOAEL	LOAEL	Reference
Rats							
344-Fischer (n= 20 per group)	Aluminium chlorohydrate	6 months (5d/wk and 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m^3	Increase in alveolar macrophages, granulomatous lesions in lung	0.065 mg Al/m^3	0.65 mg Al/m^3	Steinhagen et al. (1978)
344-Fischer (n≈ 17 per group)	Aluminium chlorohydrate	6 months (5d/wk and 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m^3	Increase in relative lung weight	M: 0.065 mg Al/m^3 F: 0.65 mg/m^3 Al	M: 0.65 mg Al/m^3 F: 6.5 mg/m^3 Al	Stone et al. (1979)
Sprague-Dawley (n= 31 per group)	Aluminium chloride	30, 60 or 90 days Water	0, 5, 50 or 500 mg Al/l	No histological changes in lungs	70 mg/kg bw per day		Dixon et al. (1979)
Sprague-Dawley (n= 10 per group)	Aluminium nitrate	1 month Water	0, 375, 750 or 1500 mg/kg bw per day	Weight and histological changes in lungs No effect was observed at the highest dose	190 mg Al/kg bw/day		Gómez et al. (1986)
Hamsters							

Strain	Aluminium compound	Duration and exposure route	Concentra-tion	Endpoint	NOAEL	LOAEL	Reference
Golden Syrian	Aluminium chlorohydrate	5 or 6 weeks (5d/wk and 6hr/d) Inhalation	10 mg Al/m³	Alveolar thickening, increased number of foci of macrophages and heterophils	-	10 mg Al/m³	Drew et al. (1974)
Guinea pigs							
Hartley (n= 20 per group)	Aluminium chlorohydrate	6 months (5d/wk and 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m³	Increase in alveolar macrophages, granulomatous lesions in lung	0.065 mg Al/m³	0.65 mg Al/m³	Steinhagen et al. (1978)
Hartley (n= 15 per group)	Aluminium chlorohydrate	6 months (5d/wk and 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m³	Increase in relative lung weight	0.65 mg Al/m³	6.5 mg Al/m³	Stone et al. (1979)

4.7.3 Haematological effects

4.7.3.1 Human data

No adverse haematological effects were noted in a group of seven workers after 6 months of exposure to aluminium fumes or dust (Mussi et al. 1984). Exposure levels ranged from 1 to 6.2 mg/m³ Al, predominantly as aluminium oxide. No other study was found on this subject.

4.7.3.2 Animal data

Some animal studies (mice, rats, guinea pigs and dogs) have been identified regarding haematological effects after subchronic exposure to aluminium compounds. These studies are described below and summarised in Table VII.53.

- **Mice**

In the study by Oteiza et al., female Swiss-Webster mice were exposed to aluminium chloride for 5-7 weeks, in the diet at doses of 3 (control) or 1000 µg Al/g diet. No alteration in haematocrit levels was observed (Oteiza et al. 1993). The NOAEL is 1000 mg/kg bw/d aluminium chloride, that is 195 mg Al/kg bw per day (the highest tested dose).

- **Rats**

Rats (Fischer-344) exposed for 6 months (5d/wk, 6hr/d) to aluminium chlorohydrate at concentrations of 0, 0.25, 2.5 or 25 mg/m³ did not present haematological adverse effects (Steinhagen, Cavender, and Cockrell 1978) leading to a NOAEL of 25 mg/m³ for aluminium chlorohydrate corresponding to 6.5 mg Al/m³ (the highest tested dose).

In the study by Stone et al., rats (Fisher-344) were exposed for 6 months (5d/wk, 6hr/d) to 0.25, 2.5 or 25 mg/m³ of aluminium chlorohydrate. At necropsy, peripheral blood was collected for haematological determinations (total red cells count, total white cells count, haematocrit, mean corpuscular volume, and total haemoglobin). The authors did not report any haematological effect following this exposure (Stone et al. 1979). The NOAEL was 25 mg/m³ aluminium chlorohydrate corresponding to 6.5 mg Al/m³ (the highest tested dose).

In the study by Gómez et al., Sprague-Dawley rats were exposed to aluminium nitrate in water for one month at doses of 0, 375, 750 or 1500 mg/kg bw per day. Hyperaemia in the red pulp of the spleen was observed in the 750 and 1500 mg/kg bw per day groups, it was also observed in the liver at the highest dose (Gómez et al. 1986). The LOAEL was 750 mg/kg bw per day of aluminium nitrate, corresponding to 95 mg Al/kg bw/day and the NOAEL was 375 mg/kg bw/d corresponding to 47 mg Al/kg bw per day.

In a study by Domingo et al., Sprague-Dawley rats were exposed to aluminium nitrate for 100 days at doses of 0, 360, 720 or 3600 mg/kg bw per day through drinking water. Rats exposed to aluminium did not show any differences in haematocrit or haemoglobin levels compared to controls (Domingo et al. 1987b). The NOAEL for haematological effects was 3600 mg/kg bw per day of aluminium nitrate, equivalent to 468 mg Al/kg bw per day (highest tested dose).

Rats (Sprague-Dawley) exposed to aluminium citrate for 8 months, through drinking water (80 mmol/L), presented decreased haemoglobin, haematocrit and haptoglobin levels, increased reticulocyte levels, and inhibition of colony-forming units-erythroid (CFU-E) proliferation compared to controls (Vittori et al. 1999). The corresponding LOAEL was 230 mg Al /kg bw per day. Anisocytosis (variation in red blood cell size), anisochromia (variation in red blood cell colour) and poikilocytosis (abnormal red blood cell shapes) were also observed in aluminium exposed rats. Furthermore, in this study, rats exposed to aluminium were not iron depleted (normal range of plasma iron concentration and total iron binding capacity) however, there was a decrease in iron uptake and iron incorporation into haem by the bone marrow cells.

In a study by Zhang et al., male Wistar rats (n=50 per group) received aluminium chloride in their drinking water for up to 150 days at 0 or 430 mg Al/L (approximatively 0 and 67-100 mg Al/kg bw/day respectively). Body weight of aluminium treated rats was significantly decreased from day 60, transferrin and total iron binding capacity were significantly higher than in the control group from day 90, and soluble transferrin receptor levels as well as erythrocyte counts were lower than controls from day 60 (L. Zhang et al. 2011). The LOAEL was 67 mg Al/kg bw per day.

- **Guinea pigs**

Guinea pigs exposed for 6 months (5d/wk, 6hr/d) to 0, 0.25, 2.5 or 25 mg/m^3 aluminium chlorohydrate did not present haematological adverse effects (Steinhagen, Cavender, et Cockrell 1978), resulting in a NOAEL of 6.5 mg Al/m^3 (the highest tested dose).

In the study by Stone et al., guinea pigs were exposed for 6 months (5d/wk, 6hr/d) to 0.25, 2.5 or 25 mg/m^3 of aluminium chlorohydrate. At necropsy, peripheral blood was collected for haematological determinations (total red cells, total white cells, haematocrit, mean corpuscular volume, and total haemoglobin). The authors did not report any haematological effect following this exposure (Stone et al. 1979). The NOAEL was 25 mg/m^3 aluminium chlorohydrate, corresponding to 6.5 mg Al/m^3 (the highest tested dose).

- **Dogs**

Beagle dogs exposed to aluminium phosphate for 6 months through their diet(dietary concentrations of 0, 0.3, 1.0 or 3.0% of sodium aluminium phosphate) had no haematological effects (Katz et al. 1984). In this study haematology, urinalysis and blood chemistry tests were performed in addition to prothrombin and activated partial thromboplastin time measurements. The NOAEL was 88 mg Al/kg bw per day. Similarly, Pettersen et al., exposed beagle dogs exposed to aluminium phosphate for 26 weeks through the diet (0, 0,3%, 1% and 3% of sodium aluminium phosphate). In this study, haematocrit, haemoglobin concentration, erythrocyte, leukocyte and platelet counts were determined but no haematological effects related to the aluminium treatment were observed (Pettersen et al. 1990). The NOAEL was 75 mg Al/kg bw per day.

Table VII.53: Animal studies on aluminium subchronic exposure haematological toxicity

Strain	Aluminium compound	Durant and exposure route	Doses	Endpoint	NOAEL	LOAEL	Reference
Mice							
Swiss-Webster (n= 10 per group)	Aluminium chloride	5-7 weeks Diet	3 (control) or 1000 µg Al/g diet	No alteration in haematocrit levels at the highest dose	195 mg Al/kg bw per day		Oteiza et al. (1993)
Rats							
Fischer-344 (n= 20 per group)	Aluminium chlorohydrate	6 months (5d/wk, 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m^3	No haematological adverse effects at the highest dose	6.5 mg Al/m^3		Steinhagen et al. (1978)
Fischer-344 (n≈ 17 per group)	Aluminium chlorohydrate	6 months (5d/wk, 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m^3	No haematological adverse effects at the highest dose	6.5 mg Al/m^3		Stone et al. (1979)
Sprague-Dawley (n= 10 per group)	Aluminium nitrate	1 month Water	0, 375, 750 or 1500 mg/kg bw per day	Hyperaemia in the red pulp of the spleen	47 mg Al/kg bw/day	95 mg Al/kg bw/day	Gómez et al. (1986)
Sprague-Dawley (n= 10 per group)	Aluminium nitrate	100 days Water	0, 360, 720 or 3600 mg/kg bw per day	Haematocrit or haemoglobin levels / No effect was observed at the highest dose	468 mg Al/kg bw per day		Domingo et al. (1987b)
Sprague-Dawley (Control: n=8; Al group: n= 10)	Aluminium citrate	8 months Water	0, 80 mmol/L	Decreased haemoglobin, haematocrit & haptoglobin levels, increased reticulocyte levels, and inhibition of CFU-E proliferation		230 mg Al /kg bw per day	Vittori et al. (1999)
Wistar (n= 50 per group)	Aluminium chloride	150 days Water	0 and 67-100 mg Al/kg bw/day	Disruption of iron homeostasis		67 mg Al/kg bw per day	Zhang et al. (2011)
Guinea pigs							
Hartley (n= 20 per group)	Aluminium chlorohydrate	6 months (5d/wk, 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m^3	No haematological adverse effects at the highest dose	6.5 mg Al/m^3		Steinhagen et al. (1978)

Strain	Aluminium compound	Durant and exposure route	Doses	Endpoint	NOAEL	LOAEL	Reference
Hartley (n= 15 per group)	Aluminium chloorhydrate	6 months (5d/wk, 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m³	No haematological adverse effects at the highest dose	6.5 mg Al/m³		Stone et al. (1979)
Dogs							
Beagle (n= 12 per group)	Aluminium phosphate	6 months Diet	0, 0,3%, 1% and 3% of sodium Al phosphate	No haematological adverse effects at the highest dose	88 mg Al/kg bw per day		Katz et al. (1984)
Beagle (n= 8 per group)	Aluminium phosphate	26 weeks Diet	0, 0,3%, 1% and 3% of sodium Al phosphate	No haematological adverse effects at the highest dose	75 mg Al/kg bw per day		Pettersen et al. (1990)

4.7.4 Bone related effects

4.7.4.1 Human data

No reliable human studies on bone related effects were identified following subchronic exposure to aluminium compounds.

4.7.4.2 Animal data

Some animal studies (rats and guinea pigs) have been identified regarding bone related effects after subchronic exposure to aluminium compounds. These studies are described below and summarised in Table VII.54.

- **Rats**

In the study conducted by Steinhagen et al., rats (Fischer-344) exposed through inhalation to aluminium chlorohydrate (0, 0.25, 2.5 or 25 mg/m³) for 6 months (5 d/wk, 6hr/d) did not present histological changes in the muscle or bone (Steinhagen, Cavender, et Cockrell 1978). The NOAEL was 6.5 mg Al/m³ (the highest tested dose).

Male STD Wistar rats exposed to aluminium lactate in the diet (1000 µg Al/g diet) for 10 weeks did not show pathological changes of bones (Konishi et al. 1996). The NOAEL was 90 mg Al/kg bw per day (the only tested dose). In a study by Li et al., four-week-old Wistar rats were exposed to 0 or 430 mg Al^{3+}/L as aluminium chloride in drinking water for 150 days. Every 30 days, 10 rats were scarified in each group. From day 60, the body weight of aluminium-treated rats was significantly lower than the control group. In the aluminium treated group, aluminium levels in bone were significantly higher, calcium and magnesium levels in bone were significantly lower from days 120-150 and, phosphorus levels were significantly lower from day 150 compared to the control group. Levels of zinc, iron, copper, manganese, boron, strontium, and selenium in bone also decreased significantly in the aluminium treated group from day 60. Finally, bone mineral density of the femur metaphysis on days 120 and 150 was significantly lower in the aluminium-treated group from day 120 (Li et al. 2011). The LOAEL is of 430 mg Al/l equivalent to 38.7 mg Al/kg bw per day.

Male Wistar rats were exposed to 0 or 0.4 g/L of aluminium chloride (resulting in doses of 0 and 64 mg/kg bw $AlCl_3$ per day) in drinking water for up to 120 days. From day 60, body weights of aluminium-treated rats were significantly lower compared to control group. Aluminium levels in serum and in the femur were significantly higher following aluminium treatment compared to controls. The bone mineral densities of the proximal and the distal femoral metaphysis were significantly lower from day 120 compared to controls and the histological structure of the bone was disrupted from day 90. Aluminium chloride exposure also inhibited the Wnt/β-catenin signaling pathway as the mRNA expression of Wnt3a, Fzd2, LRP-5, β-catenin, Tcf4, cyclin D1 and c-Myc, the protein levels of Wnt3a and β-catenin and the activities of Fzd2 and LRP-5 were decreased in the aluminium-treated rats

(X. Sun et al. 2015). The LOAEL of 64 mg/kg bw per day of aluminium chloride is equivalent to 13 mg Al/kg bw per day.

In another study by Zhang et al., male Wistar rats received aluminium chloride for 120 days at doses of 0, 0.4, 0.8 or 1.6 g/L $AlCl_3$ in drinking water (resulting in 64, 128, 256 mg/kg bw $AlCl_3$ per day). Body weights were decreased in all aluminium-treated groups compared to the control. Rats treated with aluminium had a significantly higher content of aluminium in serum and cartilage and a significantly higher level of C-telopeptide of type II collagen in serum. Serum levels of type II collagen (Col II) and alkaline phosphatase (ALP), and the mRNA expressions of TGF-b1 (Transforming Growth Factor Beta 1), BMP-2 (Bone Morphogenetic Protein 2), ALP and Col II were all decreased following aluminium treatment (Fan Zhang et al. 2017). Cartilage histological structure was also disrupted following aluminium treatment. The LOAEL is of 64 mg/kg bw $AlCl_3$ per day is equivalent to 13 mg Al/kg bw per day.

Aluminium chloride was also administered to male Wistar rats by gavage at doses of 0 or 8.3 mg/kg/day for 60 days (dose chosen to match the dietary aluminium consumption of humans per day). Results of this study showed that aluminium can induce changes in the mineral content and in the mineralised bone microstructure associated with alveolar bone loss (Souza-Monteiro et al. 2021). The LOAEL of 8.3 mg/kg bw $AlCl_3$ per day corresponds to 1.68 mg Al/kg bw per day.

- **Guinea pigs**

In the study conducted by Steinhagen et al., Hartley guinea pigs exposed through inhalation to aluminium chlorohydrate (0, 0.25, 2.5 or 25 mg/m^3) for 6 months (5 d/wk, 6hr/d) did not present histological changes in the muscle or bone (Steinhagen, Cavender, et Cockrell 1978). The NOAEL was 6.5 mg Al/m^3 (the highest tested dose).

Table VII.54: Animal studies on aluminium sub-chronic exposure musculoskeletal toxicity

Strain	Aluminium compound	Duration or exposure route	Doses	Endpoint	NOAEL	LOAEL	Reference
Rats							
Fischer-344 (n= 20 per group)	Aluminium chlorohydrate	6 months (5d/wk, 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m^3	No effect was observed at the highest dose	6.5 mg Al/m^3		Steinhagen et al. (1978)
STD Wistar (n= 4-5 per group)	Aluminium lactate	10 weeks Diet	0, 1000 µg Al/g diet	No effect was observed at the highest dose	90 mg Al/kg bw per day		Konishi et al. (1996)
Wistar (n=50 per group)	Aluminium chloride	150 days Water	0 or 430 mg Al/L	Lower bone mineral density of the femur		38.7 mg Al/kg bw per day	Li et al. (2011)
Wistar (n=80 per group)	Aluminium chloride	120 days Water	0, 64 mg/kg bw per day	Lower bone mineral density and disruption of histological structure of femora		13 mg Al/kg bw per day	Sun et al. (2015)

Strain	Aluminium compound	Duration or exposure route	Doses	Endpoint	NOAEL	LOAEL	Reference
Wistar (n=20 per group)	Aluminium chloride	120 days Water	0, 0.4, 0.8 or 1.6 g/L	Inhibition of cartilage stimulatory growth factors expressions, disruption of cartilage histological structure		13 mg Al/kg bw per day	Zhang et al. (2017)
Wistar (n=8 per group)	Aluminium chloride	60 days Gavage	0 or 8.3 mg/kg/day	Changes in the mineral content and mineralised bone microstructure, alveolar boneloss		1.68 mg Al/kg bw per day	Souza-Monteiro et al. (2021)
Guinea pigs							
Hartley (n= 20 per group)	Aluminium chlorohydrate	6 months (5d/wk, 6hr/d) Inhalation	0, 0.25, 2.5 or 25 mg/m^3	No effect was observed at the highest dose	6.5 mg Al/m^3		Steinhagen et al. (1978)

4.7.5 Other effects

4.7.5.1 Human data

No reliable human studies on systemic toxicity were identified following subchronic exposure to aluminium compounds.

4.7.5.2 Animal data

Several animal studies (mice, rats, guinea pigs and dogs) reporting systemic toxicity after subchronic exposure to aluminium compounds, have been identified. These studies are described below and summarised in Table VII.55.

- **Mice**

No effects on the body weight were observed:

- in Swiss-Webster mice exposed to 25, 500 or 1000 µg Al/g diet as aluminium lactate from Gd1 till Ld21(NOAEL of 1000 mg/g diet corresponding to 330 mg Al/kg bw per day in dams) (Donald et al. 1989);
- in Swiss-Webster mice exposed to 25 (control), 500, or 1000 µg Al/g diet as aluminium lactate over 6 weeks (NOAEL of 1000 mg/g diet, corresponding to 130 mg Al/kg bw per day) (Golub et al. 1989);
- and in Swiss-Webster mice exposed to 25 (control) or 1000 µg Al/g diet as aluminium lactate for 90 days (NOAEL of 195 mg Al/kg bw per day) (Golub et al. 1992b).

A decreased body weight in lactating mice was reported in the study of Golub et al., where mice were fed with 25 (control) or 1000 µg Al/g diet as aluminium lactate during gestation and from day 1 to day 21 of lactation (LOAEL of 250 mg Al/kg bw per day) (Golub, Keen, et Gershwin 1992a).

In the study by Oteiza et al., female Swiss-Webster mice exposed to aluminium chloride for 5-7 weeks, in the diet at doses of 3 (control) or 1000 µg Al/g diet did not have alterations in body, brain and liver weight (Oteiza et al. 1993). The NOAEL of 1000 µg/ g diet corresponds to 195 mg Al/kg bw per day (the highest tested dose).

In a study by Golub et al., Swiss-Webster mice were exposed to 6 (control) or 1025 µAl/g diet as aluminium lactate from conception to 6 months of age. Results showed that aluminium exposed mice presented a 19% increase in

spleen weights, depressed spleen cell concentrations of IL-2, INF-g and TNF-a and a deficit of CD4+ cells in T-cell populations (Golub et al. 1993). The LOAEL was 200 mg Al/kg bw per day.

Pregnant Swiss-Webster mice were exposed through the diet to 25 (control), 500 or 1000 µgAl/g diet as aluminium lactate, during gestation and lactation (6 weeks). Afterwards, dams and one male and female per litter were inoculated with *Listeria monocytogenes* and kept on the same diet for 10 days during which they were monitored. The mortality rate was significantly higher in the dams exposed to aluminium compared to the control group and the cumulative deaths were lower in mice treated with 500 µAl/g diet compared to the ones treated with 1000 µg Al/g diet. In the offspring challenged after weaning, there was no difference in the mortality between the diet groups (Yoshida et al. 1989). In the same study, virgin female mice exposed to the same amount of aluminium in diet did not show a change in susceptibility to bacterial infection, withno statistically significant difference in mortality).

- **Rats**

In the study by Steinhagen et al., no histological or organ weight changes were observed in the heart, liver, kidneys, reproductive and gastrointestinal tissues, adrenal, thyroid or pituitary glands, skin and eyes, and in the body weight of rats (Fischer-344) exposed for 6 months (5d/wk, 6hr/d) to 0.25, 2.5 or 25 mg/m^3 aluminium chlorohydrate (NOAEL : 25 mg/m^3 aluminium chlorohydrate, corresponding to 6.5 mg Al/m^3) (Steinhagen, Cavender, et Cockrell 1978).

In the study by Stone et al., no effects on the body weight of Fischer-344 rats were observed following the inhalation of 0.25, 2.5 or 25 mg/m^3 aluminium chlorohydrate over 6 months, even at the high dose exposure (Stone et al. 1979). The NOAEL was 25 mg/m^3 corresponding to 6.5 mg Al/m^3. In the study conducted by Gómez et al., no organ weight or histopathological changes were observed in the heart, kidney and gastrointestinal tissues and no effect on the body weight of Sprague-Dawley rats exposed to the highest dose of aluminium nitrate in drinking water (1500 mg/kg bw per day) over one month, equivalent to 190 mg Al/kg bw/day. In the liver, a hyperaemia was reported with periportal monocytic infiltrate at the highest dose only (Gómez et al. 1986). Thus, the NOAEL for hepatic effects was considered to be 750 mg/kg bw of aluminium nitrate equivalent to 95 mg Al/kg bw per day.

No effects on the body weight were observed in male STD Wistar rats exposed to aluminium lactate in the diet (1000 µg Al/g diet) for 10 weeks (Konishi et al. 1996) which corresponds to a NOAEL of 90 mg Al/kg bw per day.

Following oral intake of aluminium nitrate in drinking water at doses of 0, 360, 720 or 3600 mg/kg bw per day over 100 days in female rats (Sprague-Dawley), Domingo et al., reported no organ weight or histopathological changes in the heart, liver, kidney and no effect on the body weight (Domingo et al. 1987b). The NOAEL of 3600 mg/kg aluminium nitrate per day corresponds to of 468 mg Al/kg bw per day.

Rats (21 days old and 18 months old) were exposed to aluminium nitrate in drinking water at a dose of 0 or 100 mg Al/kg bw per day (citric acid was added to the water) for 100 days. A decrease in the body weight gain was reported in the aged group (M. Teresa Colomina et al. 2002). The LOAEL was 100 mg Al/kg bw per day.

Male Wistar rats (n=10 per group) were administered 0, 0.4, 0.8 or 1.6 g/L of aluminium chloride in their drinking water over a 120-day period. There was a decrease in the superoxide dismutase (SOD) activity in the kidney, significant only at the high dose group and a dose dependent decrease in the glutathione peroxidase (GSH-PX) activity in the kidney. A dose dependent increase in malondialdehyde, β2-microglobulin and cystatin C concentrations was also observed. Authors concluded that aluminium chloride induces oxidative stress and suppresses kidney function (Liu et al. 2016).

In a study by Zhang et al., aluminium chloride was intragastrically administered to male Wistar rats at doses of 0, 64.18, 128.36 or 256.72 mg/kg bw of aluminium chloride for 120 days. There was dose dependent increase in the systolic and mean arterial blood pressure (significant from the mid-dose group), an increase of osmotic fragility of the erythrocyte (significant at the high dose only), a decrease in the percentage of the membrane protein (significant from the mid dose for some and at the high dose for all), a decrease in the activities of $Na^{(+)}/K^{(+)}$-ATPase, $Mg^{(2+)}$-ATPase, $Ca^{(2+)}$-ATPase, CAT, SOD and GSH-pX (from the low dose) and an increased malondialdehyde content of erythrocyte membrane (Q. Zhang et al. 2016). In this study the NOAEL and LOAEL were 64.18 and 128.36 mg/kg bw aluminium chloride per day corresponding to 12.8 and 25.7 mg/kg bw Al per day, respectively.

Male Wistar rats, received aluminium chloride in drinking water, at doses of 0, 0.4, 0.8 or 1.6 mg/mL aluminium chloride (resulting in the doses of $AlCl_3$ at 0, 64, 128, and 256 mg/kg bw per day) for 120 days. Results showed dose-dependent histopathological lesions in the liver. Aluminium exposure reduced the electron transport chain complexes I–V activities and adenosine triphosphate (ATP) level in the liver mitochondria of aluminium-treated rats. The mitochondria DNA transcript levels (measured by ND1, ND2, Cyt-b, COX1, COX3 and ATPase6 mRNA expressions) also decreased in the liver following aluminium exposure. Accumulation of reactive oxygen species, decreased superoxide dismutase activity and increased 8-OHdG levels (8-Hydroxy-2'-deoxyguanosine is a biomarker commonly used to measure oxidative stress) in mitochondria were also observed in the aluminium group (F. Xu et al. 2017). LOAEL was 64 mg/kg bw aluminium chloride per day, corresponding to 13 mg/kg bw aluminium per day.

Male Wistar rats were administered aluminium chloride over 60 days at doses of 0 or 8.3 mg Al/kg bw per day through drinking water or over 42 days at doses of 0 or 100 mg Al/kg bw per day by gavage. There was a significant increase in systolic blood pressure in the high and low dose group at the third week (but not at the end for the low dose), an increased ROS (reactive oxygen species) production from NAD(P)H oxidase, an increase in contractile prostanoids mainly from COX-2 (cyclooxygenase-2, an enzyme involved in the production of prostaglandins) following aluminium exposure, thus, inducing vascular dysfunction and increasing blood pressure (Caroline Silveira Martinez et al. 2017a).

- **Guinea pigs**

No histological or organ weight change were observed in the heart, liver, kidneys, reproductive and gastrointestinal tissues, adrenal, thyroid or pituitary glands, skin and eyes, nor in body weight of guinea pigs exposed over 6 months (5d/wk, 6hr/d) to 0.25, 2.5 or 25 mg/m³ aluminium chlorohydrate (NOAEL = 25 mg/m³ aluminium chlorohydrate corresponding to 6.5 mg Al/m³) (Steinhagen, Cavender, et Cockrell 1978).

In the study by Stone et al., 1979, no effects on body weight of guinea pigs were observed following the inhalation of 0.25, 2.5 or 25 mg/m³ aluminium chlorohydrate over 6 months, even at the high dose exposure. The NOAEL was 25 mg/m³ aluminium chlorohydrate, corresponding to 6.5 mg Al/m³ (Stone et al. 1979).

- **Dogs**

Following dietary exposure to aluminium phosphate, the NOAEL for some systemic effects in dogs were 75 and 88 mg Al/kg bw per day, respectively in the study of Pettersen et al. (exposure for 26 weeks, no difference in organ weight of the heart, kidneys, thyroid, adrenals) and the one of Katz et al. (exposure for 6 months, no difference in organ weight or histopathological changes in the heart, liver, kidneys, pituitary, thyroid, adrenals and no ocular changes) (Katz et al. 1984; Pettersen et al. 1990).

Table VII.55: Animal studies on aluminium sub-chronic exposure systemic toxicity

Strain	Aluminium compound	Duration and exposure route	Doses	Endpoint	NOAEL	LOAEL	Reference
Mice							
Swiss-Webster (n= 16 per group)	Aluminium lactate	Gd1-Ld21 Diet	25 (control), 500 or 1000 µg Al/g diet	Body weight of dams / No effect observed at the highest dose	330 mg Al/kg bw per day		Donald et al. (1989)
Swiss-Webster (n= 5 per group)	Aluminium lactate	6 weeks Diet	25 (control), 500 or 1000 µg Al/g diet	Body weight / No effect observed at the highest dose	130 mg Al/kg bw per day		Golub et al. (1989)

Strain	Aluminium compound	Duration and exposure route	Doses	Endpoint	NOAEL	LOAEL	Reference
Swiss-Webster (n= 12 per group)	Aluminium lactate	90 days Diet	25 (control) or 1000 µg Al/g diet	Body weight / No effect observed at the highest dose	195 mg Al/kg bw per day		Golub et al. (1992b)
Swiss-Webster (Control: n= 14; Al group: n= 9)	Aluminium lactate	Gd1-Ld21 Diet	25 (control) or 1000 µg Al/g diet	Body weight of lactating mice		250 mg Al/kg bw per day	Golub et al. (1992a)
Swiss-Webster (n= 10 per group)	Aluminium chloride	5-7 weeks Diet	3 (control) or 1000 µg Al/g diet	Body, brain and liver weight / No effect observed at the highest dose	195 mg Al/kg bw per day		Oteiza et al. (1993)
Swiss-Webster (n=10-11 per group)	Aluminium lactate	Gd0- PND 180 Diet	6 (control) or 1025 µAl/g diet	Increased spleen weights, Depressed spleen cell concentrations of IL-2, IFN-g and TNF-a and a deficit of CD4+ cells in T-cell populations		200 mg Al/kg bw per day	Golub et al. (1993)
Rats							
Fischer-344 (n= 20 per group)	Aluminium chlorohydrate	6 months Inhalation	0, 0.25, 2.5 or 25 mg/m^3	Histological / weight change of heart, liver, kidneys, reproductive and gastrointestinal tissues, adrenal, thyroid, pituitary glands, skin and eyes and in the body weight / No effect observed at the highest dose	6.5 mg Al/m^3		Steinhagen et al. (1978)
Fischer-344 (n≈ 17 per group)	Aluminium chlorohydrate	6 months Inhalation	0, 0.25, 2.5 or 25 mg/m^3	Body weight / No effect observed at the highest dose	6.5 mg Al/m^3		Stone et al. (1979)
Sprague-Dawley rats (n= 10 per group)	Aluminium nitrate	1 month Water	0, 375, 750, 1500 mg/kg per day	Weight or histopathological changes in the heart, kidney and gastrointestinal tissues and body weight	190 mg Al/kg bw/day		Gómez et al. (1986)
				Hyperaemia with periportal monocytic	95 mg Al/kg bw/day	190 mg Al/kg bw/day	

Strain	Aluminium compound	Duration and exposure route	Doses	Endpoint	NOAEL	LOAEL	Reference
				infiltrate in the liver			
STD Wistar (Control: n= 5; Al group: n= 4)	Aluminium lactate	10 weeks Diet	0 or 1000 µg Al/g diet	No effect on the body weight at the highest tested dose	90 mg Al/kg bw per day		Konishi et al. (1986)
Sprague-Dawley (n= 10 per group)	Aluminium nitrate	100 days Water	0, 360, 720 or 3600 mg/kg bw per day	No weight or histopathological changes in the heart, liver, kidney and the body weight at the highest tested dose	468 mg Al/kg bw per day		Domingo et al. (1987b)
(NS) (n=16 per group)	Aluminium nitrate	100 days Water	0 or 100 mg/kg per day + citric acid added	Decreased body weight		100 mg Al/kg bw per day	Colomina et al. (2002)
Wistar (n= 20 per group)	Aluminium chloride	120 days Gavage	0, 64.18, 128.36 or 256.72 mg/kg bw per day	Increase in the systolic and mean arterial blood pressure	12.8 mg Al/kg bw per day	25.7 mg Al/kg bw per day	Zhang et al. (2016)
Wistar (n=10 per group)	Aluminium chloride	120 days Water	0, 64, 128, and 256 mg/kg bw per day	Histopathological lesions in the liver		13 mg Al/kg bw per day	Xu et al. (2017)
Wistar (n=10 per group)	Aluminium chloride	42 days High dose Gavage	0 or 100 mg Al/kg bw per day	Increase in systolic blood pressure		8.3 mg Al/kg bw per day	Martinez et al. (2017a)
		60 days Low dose Water	0 or 8.3 mg Al/kg bw per day				
Guinea pigs							
Hartley (n= 20 per group)	Aluminium chlorohydrate	6 months Inhalation	0, 0.25, 2.5 or 25 mg/m^3	No histological / weight change of heart, liver, kidneys, reproductive and gastrointestinal tissues, adrenal, thyroid, pituitary glands, skin and eyes and in the body weight at the highest dose tested	6.5 mg Al/m^3		Steinhagen et al. (1978)
Hartley	Aluminium chlorohydrate	6 months Inhalation	0, 0.25, 2.5 or 25 mg/m^3	No effect on the body weight at the highest tested dose	6.5 mg Al/m^3		Stone et al. (1979)

Strain	Aluminium compound	Duration and exposure route	Doses	Endpoint	NOAEL	LOAEL	Reference
(n= 15 per group)							
Dogs							
Beagle (n= 12 per group)	Aluminium phosphate	6 months Feed	0, 0,3%, 1% and 3% of sodium Al phosphate	No effect on organ weight or changes in the heart, liver, kidneys, pituitary, thyroid, adrenals or ocular changes at the highest dose	88 mg Al/kg bw per day		Katz et al. (1984)
Beagle (n= 8 per group)	Aluminium phosphate	26 weeks Feed	0, 0,3%, 1% and 3% of sodium Al phosphate	No effect on organ weight of the heart, kidneys, thyroid, adrenal at the highest dose	75 mg Al/kg bw per day		Pettersen et al. (1990)

4.8 Chronic toxicity

4.8.1 Neurotoxicity

4.8.1.1 Human data

Dialysis Encephalopathy

Dialysis encephalopathy was formerly observed in renal failure patients undergoing long-term haemodialysis; it resulted from aluminium-containing dialysis fluids and use of aluminium containing phosphate binders. Patients generally presented with disturbances of language, mental behaviour and movement, as well as with cognitive impairment. In severe cases coma and seizures were also observed. By treating the water to remove aluminium and discontinuing aluminium-containing drugs, this disorder is now a rarely seen, but it can still occur (Yokel et Sjögren 2022).

Occupational exposure

Epidemiological studies assessing internal aluminium dose (at least aluminium levels in whole blood, plasma, serum or urine) and cognitive impairment are described here. A total of 21 cross-sectional and 4 longitudinal studies were identified, all of which focused on occupational exposure in different aluminium industries. Epidemiological studies contained only inadequate data regarding aluminium concentration in air.

It is worth noting that most, if not all, of the studies conducted in China are carried out in the same aluminium plant, and that little information is given on the crossover of volunteers between these studies (Guo et al. 1999; He, Qiao, et Sheng 2003; Yang et al. 2015; Meng et al. 2019; S. Wang et al. 2020; Lu et al. 2021; T. Zhang et al. 2021; Shang et al. 2021; Z. Y. Zhang et al. 2022; X. Zhao et al. 2022).

Hosovski et al. (1990):

147 workers from an aluminium foundry were included in a cross-sectional study (Hosovski et al. 1990). The exposed workers had a cumulative exposure of at least 6 years (12±4.5) and a job seniority of 18.9±6.9 years, which is comparable to the non-exposed workers group (n=60). Aluminium concentrations at workplaces had been measured for each worker separately during winter and summer. Concentrations were ranging from 4.6 to 11.5 mg/m^3. Number and size of dust particles were also measured, the number of particles was of 329 to 1020/cm^3 and 65.6% of dust particles measured up to 1 µm and 26.6% 1 to 5 µm.

All workers were hospitalized for 5 days, during which time aluminium concentrations in whole blood and urine were determined (flameless atomic absorption spectrometry). Psychomotor performance was assessed using the Turners apparatus, by recording the number of errors and speed of test execution. Intellectual performance was

assessed using the Wechsler test, and a quotient of verbal intelligence, performance intelligence and total intelligence was established. The use of alcohol and psychotropic drugs in the month prior to testing was considered an exclusion criterion, and no other confounding factors were considered.

Mean whole blood aluminium concentration in exposed workers was 136.85±103.15 µg/L and mean urine aluminium concentration 45.38±55.01 µg/L, while in the unexposed group, whole blood aluminium concentration was 58.09±74.73 µg/L and urine aluminium concentration 7.25±7.82 µg/L. The high blood aluminium levels measured both in exposed workers and in controls are probably indicative of a massive external contamination of the samples (or of faulty analysis).

Aluminium-exposed workers showed significant dissociation in oculomotor coordination, prolonged complex reaction times and slower psychomotor abilities. The results of the Wechsler test of intellectual ability showed impaired memory, coding, image completion and object assembling.

Bast-Pettersen et al. (1994):

A cross-sectional study was conducted in a Norwegian primary aluminium plant (Bast-Pettersen et al. 1994). It included 22 workers exposed to aluminium (14 potroom workers and 8 foundry workers) and 16 controls from other departments of the same plant and never directly exposed to aluminium. All participants were 61-66 years-old and had been employed for at least 10 years in the plant (and exposed for at least 10 years to aluminium for participants from the exposed group). In both groups exclusion criteria were: occupational exposure to other neurotoxicants, personal history of neurological disease or diabetes.

Measurements of aluminium in serum and urine and administration of psychometric tests were performed just before or soon after retirement. The method used for aluminium analysis are not specified in the published article. Disturbances of subjective well-being were assessed by a symptom questionnaire (Q16). A comprehensive neuropsychological exploration was performed to assess psychomotor function. It included the evaluation of static steadiness, simple visual reaction time, Wechsler adult intelligence scale (WAIS) digit symbol substitution test, trail making test, Benton visual retention test, WAIS digit span, Words learning and retention, WAIS information test, WAIS similarities test, WAIS vocabulary test, WAIS picture completion test and WAIS block design test.

Mean urine aluminium levels were 12.6 µg/L in potroom workers, 9.9 µg/L in foundry workers and 7.8 µg/L in controls. The corresponding values for serum aluminium were 3.6 µg/L, 4.1 µg/L and 2.9 µg/L.
Exposed workers reported more neuropsychiatric symptoms. The results also suggest increased risk of impaired visuo-spatial organization in exposed workers but the differences with controls did not attain statistical significance, possibly due to the low numbers of exposed and control participants.

Guo et al. (1999):

A Chinese cross-sectional study compared 103 aluminium-exposed workers and 64 controls, using the WHO recommended Neurobehavioral Core Test Battery (NCTB). Exposed workers were employees of a large aluminium production plant, working in the electrolysis, smelting or welding departments for at least 5 years. Controls worked in other departments, were not exposed to aluminium and were matched on age, duration of employment, education level, drinking status and smoking status.

Urinary aluminium was measured using graphite furnace absorption spectrometry, in post-shift samples. The mean (range) levels of aluminium in the exposed and control groups were 41.8 µg/g creatinine (14-9-116.2 µg/g creatinine) and 17.7 µg/g creatinine (3.5-42.8 µg/g creatinine).

The NCTB includes a Profile of mood states (POMS) questionnaire. The mean scores of the 5 negative mood variables (tension, depression, anger, fatigue and confusion) of the POMS questionnaire were higher in workers exposed to aluminium and the difference was statistically significant for tension, depression, anger and fatigue for the older (45-60 years) participants. The scores of the psychometric tests were inconsistently impaired: significant differences between exposed workers and the referent group were observed: for the digit span test, only in 25-34 year-old participants; for the digit symbol test, only in 35-44 year-old participants; for the pursuit aiming test both in 35-44 and 45-60 year-old participants (Guo et al. 1999).

Sjögren et al. (1996) ; Iregren et al. (2001):

Iregren et al., summarized data from three different studies conducted in Sweden, in order to evaluate the effects of aluminium exposure on the nervous system in groups of aluminium workers in different industries including aluminium potroom and foundry workers (n=119), aluminium welders (n=38), and workers exposed to aluminium in the production of flake powder (n=16). Data for these groups were compared with those of mild steel welders without exposure to aluminium (n=39). Both the welders and the smelters were significantly older and had been employed for longer periods. Alcohol consumption and habits did not differ between the groups; educational level was not informed for all the groups. Measurements of aluminium concentrations in blood and urine were performed by GFAAS (detection limit < 1 μg/L). The concentrations of aluminium in urine of the welders after the shift were calculated according to an equation determined in a previous study (Ljunggren et al. 1991). All biological samples from the smelter workers were collected at least 16 hours after their latest exposure. The samples from most of the flake powder production workers were collected after 5 exposure free days. Concentrations of neurotoxic metals manganese and lead in blood were also measured as they might confound the study results.

A questionnaire on exposure was carried out in addition to other rating scales to measure symptoms and mood. Performance was assessed in all four groups by a couple of tests including: simple reaction time, finger tapping speed, finger tapping endurance, digit span, vocabulary, tracking, and symbol digit coding and the Luria-Nebraska motor scale and a board test (cylinders) for motor function assessment. Some neuro-physiological examinations have also taken place (diadochokinesometric measurements and Electroencephalography (EEG)). Age differences between the groups were controlled by forcing age into the regression before entering the group variables simultaneously. The median aluminium concentration in urine was 4.7 μg/g creatinine in the reference group and 4.2, 59.0 and 24.0 μg/g creatinine for the smelters, flake powder exposed workers and welders, respectively. In blood (not specified if whole blood, serum or plasma), median concentrations of aluminium were 1.0 (range LOD-11), 1.0 (LOD-18), 9.0 (LOD-21) and 3.0 (LOD-27) μg/L in the reference group, smelters, flake powder exposed workers and welders, respectively.

The regression analyses showed a higher prevalence of CNS symptoms for the group exposed to flake powder and the smelters compared with the steel welders. Significant group differences were found for the peg board test (cylinders), the tracking task, and the simple reaction time between aluminium smelters and mild steel welders. Workers exposed to flake powders had also a significantly different result of the tracking task compared to the reference group; their performances in the cylinder peg board test and the simple reaction time were also altered but the differences did not attain statistical significance, possibly due to a lack of power resulting of the low number of flake powder workers (n=12). In any case, there were no correlations between aluminium concentrations in urine or blood and the outcome measures. Thus, the differences between steel welders and aluminium smelters can be explained by other factors than aluminium since the groups are not comparable (Iregren et al. 2001; Sjögren et al. 1996).

Akila et al. (1999):

Akila et al., conducted a cross-sectional study among 51 asymptomatic aluminium welders, in Finland. The control group was constituted of 28 age-matched mild steel welders. The mean age of the 79 male workers was 38.4 (range 22–58). Aluminium was measured in serum and urine, using graphite furnace absorption spectrometry, with Zeeman background correction. On the basis of urinary aluminium concentrations, welders were classified into three exposure groups: reference group (n=28) <1 μmol/L (mean=0.46 μmol/L) ; < 27 μg/L (mean=12,4 μg/L), low exposure group (n=27) 1.1–4.0 μmol/L (mean=2.25 μmol/L); 27-108 μg/L (mean=60.7 μg/L) and high exposure group (n=24) >4.1 μmol/L (mean=9.98 μmol/L); > 108 μg/L (mean=269.3). Age was positively correlated with both urinary (r=0.267, p=0.017) and serum (r=0.349, p=0.002) aluminium. Alcohol consumption correlated with urinary aluminium (r=0.264, p=0.019).

A comprehensive neuropsychological exploration was performed to assess psychomotor function. Five main cognitive domains were investigated with different tests: Psychomotor functions (Finger tapping speed, Santa Ana dexterity test, Simple visual reaction time (RT)), Attention (WAIS: digit span task, WAIS-R: digit symbol substitution test, Stroop colour word test, Dual task), Verbal abilities (WAIS Similarities, Synonyms), Visuospatial skills (Embedded figures, WAIS: block design test) and Memory and learning (Wechsler memory scale (WMS): paired associates, Memory for designs, Interference recall, Similarities recall, Digit symbol recall).

Aluminium welders showed no impairment on the finger tapping, Santa Ana dexterity, simple visual reaction times, any of the verbal memory tasks, the similarities subtest of Wechsler adult intelligence scale, or the Stroop task.

An exposure-dependent impairment of the performance was observed in the welders of the high exposure group for the memory for Digit symbol substitution test (p=0.025, p=0.035 after controlling for age), Item selection time (p=0.027) and Block design (p=0.036). For Embedded figure task the effect was close to the significance threshold (p=0.055) but not significant. For memory design task, welders from the high exposure group performed lower (p=0.036) but the effect was not exposure dependent.

For the dual task, an impairment of Backward counting (p=0.016) was observed also with the higher exposure group but both in dual and single task conditions, with an exposure-dependent effect.

The authors conclude that the neuropsychological exploration showed that the effects of aluminium appeared circumscribed. Neuropsychological tasks were investigated to precise the cognitive structures impaired. It was observed that performance deficiencies were mainly detected in tasks requiring working memory, particularly those involving visuospatial information. It was also shown that such impairments were more readily found in time-limited tasks involving visually presented material, in which effective visual scanning combined with control of working memory is demanded (Akila, Stollery, et Riihimäki 1999).

Hänninen et al. (1994), Riihimäki et al. (2000):

In a Finnish cross-sectional study, the relationship between elevated internal aluminium load and central nervous system function was studied in 65 aluminium welders and a referent group of 25 mild steel welders. Aluminium was measured in serum and urine, using graphite furnace absorption spectrometry, with Zeeman background correction. Sampling was performed on Monday morning before the 1st shift of the week. In the referent group, serum aluminium ranged from 0.04 to 0.12 µmol/L (1-3.2 µg/L) and urine aluminium from 0.1 to 1.3 µmol/L (2,7-35.1 µg/L), median values were 0.08 µmol/L (2.2 µg/L) and 0,4 µmol/L (10.8 µg/L), respectively. A low and a high exposure groups of aluminium welders were defined, according to serum and urine aluminium levels: median (and range) values for serum aluminium concentrations, in the low and high exposure groups were 0.14 µmol/L (0.07-0.24 µmol/L; 3.8 µg/L (1.9-6.5 µg/L)) and 0.46 µmol/L (0.27-1.00 µmol/L; 12.4 µg/L (7.3-27 µg/L)), respectively. The corresponding values for urine aluminium concentrations were 1.8 µmol/L (0.3-5.7 µmol/L; 48.6 µg/L (8.1-153.9 µg/L)) and 7.1 µmol/L (3.2-27.3 µmol/L; 191.7 µg/L (86.4-737.1 µg/L)).

Disturbances of subjective well-being were assessed by a symptom questionnaire. A comprehensive neuropsychological exploration was performed to assess psychomotor function. Five main cognitive domains were investigated with different tests: Psychomotor functions (Finger tapping speed, Santa Ana dexterity test, Simple visual RT), Attention (WAIS: digit span task, WAIS-R: digit symbol substitution test, Stroop colour word test, Bourdon Wiesma cancellation test, Dual task), Verbal abilities (WAIS Similarities, Synonyms), Visuospatial skills (Embedded figures, WAIS: block design test) and Memory and learning (WMS: paired associates, Memory for designs, Homogeneous interference, Similarities recall, Digit symbol recall). Quantitative electroencephalography was also recorded, and P300 auditory event-related potentials were studied.

There was an exposure-related increase in reported fatigue, mild depression, and memory or concentration problems. Neuropsychological testing revealed exposition-related impairments of cancellation accuracy in the Bourdon-Wiesma test, of backwards counting, of both components of the dual task, of synonyms and of memory for designs; those impairments were detectable in both the low and high exposure groups and increased with the exposure. Significant, age-adjusted correlations were observed between impairments in Digit symbol test, counting backwards, Dual task cancellation speed, and Dual task counting speed, on one hand and urine aluminium concentration on the other hand. Visual EEG analysis revealed mild diffuse abnormalities only in aluminium welders. No statistically significant differences were observed between the groups in the quantitative analysis. No significant association was observed between serum or urine aluminium concentration and P300 amplitude or latency (Hänninen et al. 1994; V. Riihimäki et al. 2000).

Bast-Pettersen et al. (2000):

In a Norwegian cross-sectional study, 20 aluminium welders (mean age 33 years), who had been exposed to aluminium for an average of 8.1 years, were tested for tremor and simple reaction time and screened for

neuropsychiatric symptoms. They were compared with 20 construction workers matched for age. Exclusion criteria in both groups were diseases affecting the CNS and exposure to solvents.

Urinary aluminium was measured using graphite furnace absorption spectrometry, with Zeeman background correction, in pre-shift and post-shift samples. Subjective symptoms were recorded by means of the self-administered Q16 questionnaire. Hand steadiness was measured by the Klove-Matthews Static Steadiness Test (SST). Reaction times were measured by two computerized tests (Simple reaction time and Continuous performance test).

The median (range) urinary aluminium concentrations were 0.15 µmol/mmol creatinine (0.06-0.43 µmol/mmol creatinine; 35.8 µg/g creatinine; 14.3-109.9 µg/g creatinine). Urinary aluminium was not measured in controls.

Aluminium welders reported significantly more symptoms than referents did. Although they globally performed better than referents on the tremor test, years of exposure (but not age) was associated with poorer performance (Bast-Pettersen et al. 2000).

Letzel et al. (2000):

A longitudinal study consisting of two successive cross-sectional studies was conducted at an aluminium powder-producing plant to evaluate possible exposure-related nervous system effects. In the first examination, 32 workers exposed to aluminium dust were compared to a control group of 30 unexposed persons; groups did not differ in age, sex, level of education or professional training. No exclusion criteria are reported. Five years later, in the second examination, only 21 of the exposed workers and 15 controls agreed to continue the study; this selection led to a difference in age and educational level between the two evaluations. Assessments mainly included biomonitoring, standardized medical history, neuropsychological tests (vocabulary test, three subtests of the Wechsler Adult Intelligence Survey (digit span, digit symbol, and block design), the trail making test, the syndrome short test, and a visual discriminative reaction task. An event-related P300 potential was also measured. The methods for aluminium measurements in plasma and urine are not presented in the article.

There was no concurrent intake of aluminium-containing medications, but two individuals in the initial assessment had a history of drug abuse or brain contusion, and high alcohol consumption was reported in some individuals in both groups.

Internal aluminium levels were significantly higher in the exposed group in both assessments (sampling time and analytical method not specified). Notably, in the first examination, median urine aluminium levels were 87.6 µg/g creatinine in exposed workers vs 9.0 µg/g creatinine in the control group, with median plasma aluminium of 8.7 µg/L in the exposed group versus 4.3 µg/L in the controls. In the second examination, median urine aluminium levels were 19.8 µg/g creatinine in exposed workers vs 4.5 µg/g creatinine in the control group, with median plasma aluminium of 6.7 µg/L in the exposed group vs 4.3 µg/L in the controls (with no significant difference in this latter group). The difference between both examinations is explained by the improved occupational hygiene. Regarding the psychometric tests and the P300 potentials, there was no significant exposure-related differences in any of the two cross-sectional studies. There was also no dose-response relationship between plasma or urinary aluminium concentrations, or aluminium exposure length on one hand and psychometric or P300 parameters, on the other hand (S. Letzel et al. 2000).

Polizzi et al. (2002):

A cross-sectional case-control study was carried out in 64 former Italian aluminium dust-exposed workers and in 32 unexposed controls. All participants were retired for at least 10 years. The control group included workers (from other industries), with a similar profile of age, education level, socio-economic status and clinical features. Subjects taking aluminium-containing drugs or drugs acting on the central nervous system, with kidney problems, or with a history of head trauma or psychological, sleep or neurological disorders, were excluded.

Serum aluminium was measured by GFAAS. Serum concentrations of copper and zinc and whole blood concentrations of iron, lead and manganese were simultaneously assessed. The cognitive assessment included a standardized occupational and medical questionnaire and the MMSE (Mini-Mental State Examination) test and, the CDT (Clock Drawing Test, testing visuospatial, abstraction, language and memory abilities). Auditory evoked event-related potentials (ERP-P300) were also measured.

There was a significant difference (p<0.001) between the mean serum aluminium concentrations in the workers' group (14.1 ± 3.50 µg/L) and the control group (8.2 ± 1.17 µg/L). It should be noted that blood iron levels were also higher in the workers' group. The results of the neuropsychological tests were also significantly different between the 2 groups, with a negative correlation between serum aluminium and the MMSE, MMSE-AE (adjusted for age and education) and CDT scores, and a positive correlation between serum aluminium and the MMSE and CDT times, confounders being taken into account. ERP-P300 latency was also found to correlate positively with serum aluminium concentration (Polizzi et al. 2002).

He et al. (2003):

In a cross-sectional study, He et al. (2003) studied neurobehavioural parameters, autonomic nervous function and lymphocyte subsets in 33 workers from a Chinese aluminium plant and 34 controls from a flour mill. Exclusion criteria were history of neurological disease, heart disease, hypertension, diabetes or renal disease, alcohol consumption ≥500 mL/week or cigarette smoking ≥ 40/j.

Urinary aluminium was measured using graphite furnace absorption spectrometry, in pre-shift samples. Neurobehavioral test battery included a standardized questionnaire on mood state, and psychometric tests: Simple reaction time measurement, digital symbol test, Santa Ana dexterity test, digital span test, Benton visual retention test, and Pursuit aiming.

Mean urine aluminium concentrations in the exposed workers and the control group were 40.1 µg/g creatinine and 26.8 µg/g creatinine, respectively. Reaction time was significantly slower in exposed workers. Also, the scores of the digital symbol test, the pursuit aiming were significantly lower in the aluminium-exposed group (He, Qiao, et Sheng 2003).

Buchta et al. (2003) - Kiesswetter et al. (2009):

A longitudinal study, involving aluminium welders from a car-body construction industry in Germany who were not exposed to other possible neurotoxic substances, was conducted over 4 years during which three examinations were carried out, separated by 2 years, in 1999, 2001 (M. Buchta et al. 2003) and 2003 (Ernst Kiesswetter et al. 2009). A total of 98 aluminium welders in 1999, 97 in 2001 and 92 in 2003 were compared to a demographically similar control group of 50 subjects from the same industry (age, level of education and level of carbohydrate-deficient transferrin in plasma). At the first examination, included subjects had at least 2 years of Al-welding time. Subjects with neurological diseases not due to the exposure, cerebrovascular diseases, diabetes, head injuries, insufficient knowledge of the German language were excluded.

Aluminium concentrations were measured in personal air, as well as in plasma and in urine, in pre-shift and post-shift samples, by GFAAS. Workers were examined during the day shift between 08:00 and 13:00 h, only if they had worked on the morning or afternoon shift the week before. Neurobehavioral assessments used a standardized interview, the Standard Progressive Matrices test (SPM) (only in the 1st examination), a verbal intelligence test (WST), and the European neurobehavioral evaluation system (EURO-NES) (these 3 tests, only for the last 2 examinations), a simple reaction time test, the block design test, the trail making test, four psychomotor performance tests (testing for steadiness, line tracing, aiming and tapping, and a recall of digits test (HAWIE) (the last test, performed in the 3 examinations).

There was no significant difference of total dust load when welding aluminium between the three examinations (p= 0.35), the same applies for the pre- and post-shift internal Al-loads in exposed welders across examinations. In welders, median (min, max) aluminium urine concentration (post-shift) was 37.87 (7.0–120.5) µg/g creatinine (1999), 33.57 (9.0–230.11) µg/g creatinine (2001) and 15.4 (0.7-94.9) µg/g creatinine (2003). Median plasma aluminium (post-shift) was 8.3 (2.3–42.3) µg/L (1999), 4.1 (0.72–11.7) µg/L (2001) and 4.3 (1.8-15.6) µg/L (2003). Median (min-max) aluminium urine concentration in control group was 5.2 (1.7-30.3) µg/g creatinine (1999), 6.0 (1.6-390.0) µg/g creatinine (2001) and 5.0 (0.2-40.3) µg/g creatinine (2003), and median (min-max) plasma concentration was 4.4 (1.4-31.6) µg/L (1999), 2.3 (0.7-5.9) µg/L (2001) and 3.8 (1.6-10.0) µg/L (2003). Welders and controls did not report significantly more symptoms in the modified questionnaire Q16. Furthermore, no significant differences in psychomotor performance and other neurobehavioral tasks, except for reaction time, were seen between welders and non-welders. Aluminium welders were slightly slower than controls in their reactions (decision time) but quicker in their motor movements. The corresponding multivariate analysis of

covariance for repeated measurements included both test parameters in one model. This model indicates a significant group difference (p= 0.015). There was a significant influence of age (p<0.001). As the only abnormal results in all 3 examinations were for simple reaction time and puzzling (the lower performance in one test component being compensated by better performance in the other test component), as these effects did not increase with exposure duration and as confounding factors were present, the authors conclude that their study shows no adverse neurobehavioral effect of aluminium exposure.

Buchta et al. (2005); Kiesswetter et al. (2007):

In a longitudinal study (Mark Buchta et al. 2005; E. Kiesswetter et al. 2007), exposure and neurobehavioral data of 44 aluminium exposed male welders and 37 controls from the same five German companies in the train and truck construction industry were examined for 4 years (1999, 2001 and 2003) and compared. Pre-shift and post-shift aluminium measurements were performed in the plasma and urine, using graphite furnace absorption spectrometry (GFAS). Neurobehavioral assessments used standardized interview, physical examination, a verbal intelligence test (WST), the German version of Q16 questionnaire; a recall of digits test, a block design test, a computerized test battery for motor performance, a simple reaction time measurement, a German version of the standard progressive matrices test, a trail making test, and the EURO-NES.

In welders, median urinary and plasma post-shift levels were 130 µg/L or 97 µg/g creatinine (n= 31) and 11.6 µg/L (n= 31) in 1999, 145.5 µg/L or 143.9 µg/g creatinine (n= 25) and 14.3 µg/L (n= 25), in 2001, and 93.7 µg/L or 64.5 µg/g creatinine (n= 20) and 13.2 µg/L (n= 20) in 2003. The corresponding mean values were: 210 µg/L or 135.5 µg/g creatinine and 14.8 µg/L in 1999; 191.5µg/L or 153 µg/g creatinine and 18.6 µg/L in 2001; 155.7 µg/l or 113.5 µg/g creatinine and 17.8 µg/L in 2003. In the control group, median values were 5.8 µg/L or 4 µg/g creatinine in urine and 3.5 µg/L in plasma, in 1999; 6 µg/L or 4.5 µg/g creatinine in urine and 2.8 µg/L in plasma, in 2001; 8.3 µg/L or 8.5 µg/g creatinine in urine and 4.5 µg/L in plasma, in 2003.

In 2003, the aluminium welders who had been working in this profession for an average of 15 years had no increased symptom level. The only significant difference between welders and controls was observed for block design test scores, the welders revealing significantly lower scores than controls. It should be observed that verbal IQ (WST), performances in the SPM test, in the trail making test, in the line tracing test and in switching attention tasks were also lower in welders though the intergroup differences were not statistically significant. Regression and covariance analyses showed no correlation between biomonitoring parameters and cognitive performance variables. As only 20 welders and 12 controls were included in the 2003 analysis, these negative (statistically non-significant) results could be due to the low power of the study. The authors also discuss the possibility of a healthy worker effect: workers developing symptoms might have left the plant. As only mild effects were reported, it is an unlikely hypothesis.

Giorgianni et al. (2014):

Giorgianni et al., looked for an association between serum aluminium level and cognitive impairment in 86 male aluminium welders from an Italian shipyard and in 90 “controls” from the administrative department of the same company. Serum aluminium concentration was measured using atomic absorption spectrometry. Serum concentrations of chromium, lead manganese and zinc were simultaneously measured. Neuropsychological evaluation used the WMS (form I), the Colour-Word test (Stroop test), the Attention Matrixes test. Mean ages of controls and exposed welders were 38.29±7.14 years and 38.45±6.34 years, respectively.

All participants were non-smokers. Duration of aluminium exposure was 15.79±6.50 years in welders. Mean serum aluminium concentration was 24.19±9.99 µg/L in welders and 6.93± 1.95 µg/L in controls. The authors used a nonparametric test combination (NPC) to compare the stratified volunteers between welders and controls. The stratification was done at two levels, age and length of service, using 38 years and 22 years respectively as threshold values. Decreased performances were observed in all cognitive tests for the welders group, the WMS test and the Stroop test showing a greater sensitivity than the Test of Attention Matrixes, according to the authors. Only “partial” results of the NPC test should be considered as combined values seems to overestimate impact of aluminium exposure on neuropsychological evaluation, in the attention Matrixes test, attention deficit is observed only for individuals over 38 years old and with more than 22 years of service (Giorgianni et al. 2014).

Yang et al. (2015):

Yang et al. (2015) assessed the association of cognitive impairment and aluminium exposure in 366 aluminium potroom workers (age 40-60 years old and 21.2±6.5 years of exposure time). The exclusion criteria were the consumption of aluminium-containing or psychotropic drugs, a personal history of mental or neurological disorders or of any severe disease. People with poor vision or hearing were also excluded. Serum aluminium concentration was measured using GFAAS (LOD: 1 µg/L). Cognitive function was assessed with the MMSE. Mild cognitive impairment (MCI) was diagnosed using MMSE scores (cut-offs not specified) followed by confirmation from "professional clinicians".

Median serum aluminium concentration was 48.99 µg/L (range 6.63-158.8 µg/L). Analyses were conducted after distributing the participants in three groups based on 25th and 75th percentiles of serum aluminium concentration, 0-34.02 µg/l, 34.03-61.42 µg/l and ≥ 61.43 µg/l respectively. The total MMSE score decreased when serum aluminium concentration increased. There were 43 (/366) MCI cases and their rate increased with serum aluminium concentration ($p<0.001$). The association between aluminium exposure and the risk of MCI was assessed using a logistic regression model, with adjustment for possible confounders (age, education, smoking, and drinking). Cognitive impairment was significantly associated with high aluminium exposure (OR = 2.57; IC95% 1.5-4.41) (Yang et al. 2015).

Meng et al. (2019):

A large-scale case study was conducted in an aluminium factory in China. It included 853 male workers provided with protective equipment, excluding those with a history of cognitive problems or any disease that might induce them, as well as any family history of dementia or those taking aluminium-containing medication or psychotropic drugs, as well as those using cookware or consuming fried food daily; people with poor vision or hearing were also excluded.

This study comprised of a biomonitoring study measuring plasma aluminium by ICP-MS (detection limit of 0.39 µg/L) as well as a 2-step questionnaire to assess cognitive impairment; the first stage being the MMSE and CDT tests. To increase the sensitivity and specificity of diagnosis, cognitive impairment was defined as a MMSE score of 26 or less (of 22 or less in workers whose education level is less than middle school, of 19 or less in workers whose education level is under primary school) or a CDT score of 2 or less. Participants with low MMSE or CDT scores and those complaining of memory impairment were referred to neurologists of the local University hospital for further explorations.

In all, 334 workers passed phase 2, of whom 53 (39.16%) were diagnosed with MCI, mainly on delayed recall (81.13%) and visuospatial executive ability (56.60%). For each case, 4 controls were matched by age randomly with no difference in marital status, income, smoking or drinking status; the mean age for cases and controls were between 45.04 and 44.71 years, respectively. The median (P25-P75) plasma aluminium concentration in the 53 MCI patients (18.17 (10.39, 34.96) µg/L) was significantly ($p=0.001$) higher than in the controls (12.02 (6.35, 20.86) µg/L). People with MCI were found to have a lower level of education ($p=0.001$). Conditional logistic regression was used to explore the influential factors of mild cognitive impairment. This showed that a high level of plasma aluminium increased the risk of cognitive problems (AOR= 2.24 95% CI 1.17-4.26 $p=0.014$), whereas a more advanced educational level was more of a protective factor (AOR: 0.36 95% CI 0.18-0.7 $p= 0.003$) (Meng et al. 2019).

Mohammed et al. (2020):

A cross-sectional study was conducted in an Egyptian aluminium foundry and included 75 exposed workers who wore gloves only, without masks or other protective equipment, and 75 "unexposed" controls from the administrative department of the same plant, to study the effect of exposure on cognitive performance. The two groups were matched in terms of age, gender, socioeconomic status, demographics and habits affecting health. People taking aluminium-containing or psychotropic drugs and those with a personal history of mental or neurological disorders were excluded. Serum aluminium level was measured (together with serum levels of lead, manganese and zinc), using Atomic Absorption Spectrophotometry (AAS) (LOD: 0.1 µg/dL). Serum free tau protein was also measured, using the Western blot technique. This protein is commonly found in the axons of neuronal cells and may be released into peripheral blood during neuronal damage. Cognitive functions were assessed using the Montreal cognitive assessment Basic (MoCA) test. The cutoff value retained for the definition of mild cognitive impairment was 24(/30). Occupational stress was also assessed using the Perceived stress scale (PSS14) in its

Arabic version. Quality of sleep was evaluated by the Arabic version of the Pittsburgh sleep quality index (PSQI). The Beck depression inventory (BDI) was administered to detect depression.

The exposed group had a significantly ($p<0.001$) higher serum aluminium level (mean ±SD: 0.56 mg/L ± 0.18) than the control group (0.36 mg/L ± 0.11), as did the tau protein (1.53 ± 0.58 for exposed vs. 1.03 ± 0.44 for workers, $p<0.001$). The very high serum aluminium levels measured both in exposed workers and in controls are probably indicative of a massive external contamination of the samples (or of faulty analysis).

The MoCA test showed a significantly lower performance in the exposed group (score 24.4 ± 3.4 vs 28.4 ±1.3, $p<0.001$). Regression analysis showed that cognitive performance was negatively correlated with serum aluminium and tau protein levels ($r= -.341$ and $p < 0.003$, $r=-0.250$ and $p < 0.03$ respectively). Exposed individuals were more stressed than controls, but this had no impact on cognitive performance. It should be noted that there was co-exposure to other metals, since serum lead and manganese levels were also higher in workers than in controls. As a massive external contamination of the serum sample is probable, the reported results should be considered with caution (Mohammed et al. 2020).

Wang et al. (2020):

Wang et al. (2020) conducted a cross-sectional study in 831 male workers (20-59 years old) exposed to aluminium in an aluminium factory (at least one year). Exclusion criteria were all causes of cognitive impairment, family history of neurodegenerative disease, long-term treatment with aluminium-containing drugs, poor vision or hearing. Plasma aluminium level was measured by ICP-MS. The participants were categorized into four quartiles based on their plasma aluminium concentrations, specifically, 0-8.28 µg/l (Q1), 8.28-15.26 µg/l (Q2), 15.26-27.02 µg/L (Q3), and ≥27.02 µg/L (Q4) subgroups. Cognitive function was measured using the MMSE and the CDT. Multidomain cognition was assessed through sub-tests of the MMSE and the CDT. There was no statistical difference in MMSE scores between groups.

When adjusted for age, education, income, marital status, type of work, and smoking and drinking habits and stratified by age (threshold of 40 years), a positive association was observed between plasma aluminium concentration and the risks of global cognitive impairment and multi-domain cognitive impairments. Considering total CDT scores the results were as follow: Q3 vs Q1 (OR=2.26, IC95% 1.25-4.11) and Q4 vs Q1 (OR=3.49, 1.85-6.59) but only for individuals older than 40 years. The same association was observed for executive/visuospatial abilities (OR Q3 vs Q1 = 1.77, IC95% 1.03-3.04; OR Q4 vs Q1 = 2.44, IC95% 1.35-4.41) and CDT-position errors in individuals over 40 years old (OR Q3 vs Q1 = 1.75, IC95% 1.00-3.09; ORQ4 vs Q1 = 2.22, IC95% 1.22-4.10) (S. Wang et al. 2020).

Lu et al. (2021):

Lu et al. conducted a longitudinal study that included 2 cognitive evaluations one carried out in 2014 and then another after 2 years. A measurement of plasma aluminium concentration was performed using an ICP-MS (detection limit of 0.39 µg/L and standard deviation of 0.03%-0.08%) on 276 men workers in an aluminium plant in northern China. Log10 transformed plasma aluminium concentrations were used in this analysis. All workers used the same protective equipment and were exposed to aluminium metal and fluoride salt during the process of electrolytic aluminium with no other metal exposure. Information on background and health was collected by means of an employee health questionnaire, and status on smoking, alcohol consumption, age and sex, level of education and work history as well as diseases were collected. The cognitive assessment was established by an internationally recognized cognitive test questionnaire and the following tests: MMSE, VFT (Verbal Fluency Test), SRT (Simple Reaction Time), FOME (Fuld Object Memory Evaluation, evaluating delayed memory ability), DST (Digit Span Test, testing auditory linguistic memory ability), CDT, testing visuospatial, abstraction, language and memory abilities).

Participants were divided into 3 tertiles according to plasma aluminium concentration: T1 (<17.6 µg/L) T2 (17.6-37.3 µg/L) T3 (≥37.3 µg/L). No significant differences were found among participants in terms of age, education, smoking and drinking status, or marital status. After adjusting for covariates, there was a reduction in the FOME (2014) and MMSE (2016) scores with increasing tertiles of plasma aluminium concentrations. Also, there was negative association between plasma aluminium concentration and most of the cognitive scores in 2014 and 2016. However, this association was statistically significant only for MMSE and FOME scores, in 2016. In 2016 each 10-

fold increase in P-Al concentration was significantly associated with a 0.53-point decrease in MMSE score (p = 0.002) and a 0.93-point decrease in FOME score (p = 0.008). For each 10-fold increase in P-Al concentration, there was a 0.38- point decrease in MMSE score 2016-2014 (P = 0.044) and a 1.20-point decrement in FOME score 2016-2014 (P = 0.001). Concerning the average annual change, it was statistically different for the MMSE and FOME with the P-Al increase (P < 0.05): MMSE scores declined in each tertile, with annual decline rates of 0.58%, 0.61%, and 1.84%, respectively. The decrease in FOME scores only appeared in the T2 and T3 groups, which were 0.34% and 3.33%, respectively. The trend test indicated that there was a dose-response relationship between the P-Al concentration and the MMSE score (P = 0.009) but no relationship with the FOME score (P > 0.05) (Lu et al. 2021).

Xu et al. (2021):

A cross-sectional study was conducted in 2014 at an aluminium plant in China. Ultimately, 1,660 workers were included in this study after eliminating those who had worked for < 1 year, those taking aluminium-containing medications, those with neurological and/or mental disorders, and those with no biomonitoring results. For these 1,660 workers, plasma aluminium concentration was determined by ICP-MS with a detection limit of 0.39 µg/L. Cognitive function was assessed by the following 6 tests: the MMSE, the CDT, Digit span test (DST) forward (DSFT) and backward (DSBT), FOME, VFT, SRT. Multiple linear regression analyses were used to study the correlation between plasma aluminium concentrations and cognitive function, while taking into account age, education, marital status, smoking and alcoholism, and total working hours.

All participants were male. Their average age was 40.42 ± 7.58 years; the average working duration was 17.74 ± 9.04 years. The median plasma aluminium concentration was 34.5 (P25, P75 =15.0, 42.3) µg/L which enabled the participants to be divided into 4 quartiles, Q1 (Al<15.00 µg/L), Q2 (15.00 ≤ Al<34.52 µg/L), Q3 (34.52 ≤ Al<42.25µg/L), and Q4 (Al≥42.25 µg/L). Similarly, participants were divided into 2 further categories: younger group (<40 years) and middle-aged group (≥40 years). Scores of the different tests: recall, DST, DSBT, FOME, VFT, ATIME, FAS, and SLO were significantly lower in the middle-aged group than in the younger group (p<0.05). Multiple linear regression analysis after adjusting for age, education level, marital status, smoking status, alcohol status and total working time, showed that DST and DSBT test scores were negatively correlated (p<0.05) with plasma aluminium concentration in all participants (even when looking at younger and middle-aged groups). The same negative association was found in Q3 and Q4 of the younger group, whereas in the middle-aged group it was only present in Q4 (p<0.05).

Logistic regression analysis showed that the ORs for cognitive impairment for the DSBT and DST tests for category Q4 in all participants were 9.216 (95% CI, 5.068-16.756) and 2.309 (95% CI, 1.587-3.359), respectively, they were 7. 644 (95% CI, 3.846-15.192) and 1.695 (95% CI, 1.062-2.705), respectively, in the middle-aged group, and 15.308 (95% CI, 4.180-56.059) and 3.270 (95% CI, 1.615-6.620), respectively in the younger group (S.-M. Xu et al. 2021).

Zhang T et al. (2021):

Zhang et al.. studied the effect of aluminium on cognition. In this study, they collected information from a public health surveillance project in Zhejiang (China) from which they selected 539 aluminium occupationally exposed participants (miners and workers from related factories): mean duration of exposure of 13.2 (± 11.3) years) and a control group of 1720 unexposed participants from another district. Cognitive function was measured by the MMSE. No aluminium measurement in blood or urine was conducted and, no information about personal or familial history of neurological diseases were brought up in the survey. Socio-demographic factors were compared between both groups, it is to be noted that the exposed group was younger than the unexposed one.

People exposed to aluminium had a significant (p<0.001) lower performance in the MMSE than the control group (mean score of 22.95 for unexposed vs 21.34 for exposed) and a higher risk of cognitive impairment[35]. A logistic regression model showed that aluminium-exposed group had 6.77 times more risk of cognitive impairment than the unexposed group (p<0.001), adjusted for age, sex, and educational level. The prevalence odds ratio remained high when adjusted for more covariates within another model (8.21, p<0.001). The analysis by logistic regression (covariates included age, sex, and education), showed no significant association between occupational exposure duration and cognition (p = 0.232) (T. Zhang et al. 2021).

[35] Not defined in the publication. Probably MMSE score under 26.

Shang et al. (2021):

The cross-sectional study by Shang et al., aimed to assess the relationship between the plasma aluminium levels and cognitive impairment in 187 aluminium workers from departments involved in aluminium electrolysis (cryolite-alumina molten salt electrolysis in particular). A total of 255 participants was considered for the study and 187 were selected according to the inclusion and exclusion criteria. People with known mental or neurological disease, or with a history of neurodegenerative disease in their immediate family members, or with long-term consumption of psychotropic drugs or of aluminium-containing antacid drugs were excluded, as well as those using aluminium cookware, or with an exposure to strong noise. All the Al workers considered for the study were male workers, had been exposed for more than one year and had an average age of 40.16 ± 7.73 years. Cognitive function was assessed using the Chinese version of the MoCA, which asses the following performances: 1) executive/visuospatial abilities; 2) naming; 3) attention and calculation; 4) language; 5) abstraction ability; 6) recall; 7) orientation. Results were expressed as a global score, which could have a maximal value of 30, and a score fewer than 26 points was considered as MCI. Plasma aluminium levels were quantified using ICP-MS; plasma levels of eight other elements (chromium, cobalt, copper, iron, lead, lithium, manganese, and zinc) were simultaneously measured.

Participants were divided into two groups based on their MoCA scores, consisting of 49 individuals classified as normal (MoCA score ≥26) and 138 individuals with mild cognitive impairment (MoCA score < 26). Median (IQR) plasma aluminium concentrations were 55.862 (38.701–77.012) μg/L for the normal group and 72.794 (42.510–102.652) μg/L for the MCI group. To estimate the relationship between plasma aluminium concentration and MoCA scores while adjusting for confounding factors, the study employed a multivariate generalized linear regression model and Bayesian kernel machine regression (BKMR). The results of the multivariate generalized linear model indicated a significant inverse relationship between plasma aluminium concentration and MoCA total scores (β (95% CI) -0.07 (0.108, -0.032); $p < 0.001$), after adjustment for age, BMI, education level, monthly income, marital status, working duration, shift work, smoking status, drinking status, sleep quality, physical activity, and all other plasma elements concentrations (Shang et al. 2021).

Zhang Z et al. (2022):

The study aimed to analyse the correlation between Quantitative Susceptibility Mapping (QSM) values, MoCA scores, and plasma aluminium levels in 53 Al workers issued from the previous cohort study of Shang et al. (2021). The 53 included workers were male, with an age range of 37-57 years, and were separated into two groups according to the MoCA score. Twenty-eight (28) workers were from the MCI group (MoCA score <26) and 25 from the "normal" group (MoCA score ≥26). There was no difference in age and educational level, between the two groups. Mean plasma aluminium level was higher in the MCI group (43.8 μg/L vs 33.1 μg/L). QSM values of left hippocampus, left dentate nucleus, right substantia nigra, and left putamen were higher in the MCI group compared to the "normal" group ($p<0.05$). No correlation was found between QSM values and plasma aluminium levels, suggesting that the pathological MCI mechanism is not related to plasma aluminium concentration and aluminium deposition in brain tissue. The authors concluded that, although QSM might be a valuable diagnostic marker for the diagnosis of MCI, no correlation was identified between plasma aluminium levels and QSM in Al workers (Z. Y. Zhang et al. 2022).

Zhao X et al. (2022):

In a cohort study including 352 workers from an electrolytic aluminium workshop (19-55 years old), Zhao et al. (2022) investigated neurocognitive impairment using neuropsychological tests. Exclusion criteria were: age ≥ 60 years, exposure duration < 1 year, education level lower than primary school, familial history of neurodegenerative disease, personal history of neurological or mental disease, long-term use of psychotropic drugs, consumption of aluminium-containing drugs or use of aluminium cookware, exposure to strong noise, and hearing deficiency. The tests performed included MMSE, VFT, FOME (evaluating delayed memory ability), DST (testing auditory linguistic memory ability), CDT (testing visuospatial, abstraction, language and memory abilities).

Plasma aluminium concentration was measured using GFAAS. Individuals were distributed into 4 quartiles according to plasma aluminium level (Q1 <17.7 μg/l; Q2 17.7-27.85 μg/l; Q3 27.85-41.04 μg/l; Q4 ≥ 41.04 μg/l). Participants from Q3 and Q4 groups performed poorly to cognitive function tests compared to Q1 participants (DST, DSB, VFT for Q3vsQ1 and DST, DSF, DSB, VFT for Q4vsQ1). After adjustment for age, years of employment,

education, income, smoking and drinking status, a significant association was observed between plasma aluminium concentration and a global score of cognitive impairment; it was statistically significant only for Q4 compared to Q1 participants, OR=6.172 (IC95% 2.31-16.488). With each 1μg/L rise in plasma aluminium concentration, there was a 1.051-fold increase in the risk of cognitive impairment (95% CI: 1.031 to 1.072) (X. Zhao et al. 2022).

Zhang Y et al. (2022):

Zhang et al., studied a cohort of 392 male workers from an electrolytic workshop in China. Participants have worked for 15±9 years in aluminium plant. All participants had been exposed for more than one year. People with long-term consumption of aluminium-containing antacid drugs were excluded, as well as those using aluminium cookware, or those exposed to aluminium less than one year, or those with high blood pressure or hypotension. Plasma aluminium levels were determined by ICP-MS. Thirty minutes after their blood pressure measurement, participants were interviewed to collect the cognitive function test data. Cognitive functions were assessed using several tests as the MMSE, the VFT, the Average Reaction Time (ATIME), the FOME (evaluating delayed memory ability), the DST (testing auditory breadth and auditory linguistic memory ability), the CDT (testing visuospatial construction, abstract thinking, language and memory abilities). The authors conducted a generalized linear regression model to analyse the relationship between plasma aluminium levels, cognitive functions and blood pressure.

The participants were divided into four groups based on median and quartiles of plasma aluminium levels: Q1 (< 18.08 μg/L), Q2 (18.08-28.21 μg/L), Q3 (28.21-40.88 μg/L), Q4 (≥ 40.88 μg/L). When aluminium concentration was used as a continuous variable, after adjustment for age, education level, marital status, smoking and drinking status, BMI, duration of employment, and family history of hypertension, for every e-fold increase, the MMSE, VFT, and FOM scores decreased by 1.275, 4.289 and 0.879 units respectively. When the Q4 group was compared to the Q1 group, plasma aluminium increase was associated with an increased ATIME. Plasma aluminium increase was also associated with an increasing risk of hypertension (odds-ratio, OR = 1.630, 95% CI: 1.103–2.407), and with an elevated systolic (OR = 1.578, 95% CI: 1.038–2.399) and diastolic blood pressure (OR = 1.842, 95% CI: 1.153–2.944) (Y. Zhang et al. 2022).

Zhang F et al. (2023):

In the study by Zhang et al., the relationship between cognitive impairment and occupational exposure to aluminium, as indicated by plasma levels, was examined. The participants were 54 subjects who were native Chinese male individuals, aged 37-57 years and who have worked in an aluminium factory for a minimum of 10 years. Exclusion criteria were left-handedness, history of major illness, contraindication of MRI scanning, and medical history of current serious medical problems

MoCA and verbal memory evaluated with the Rey Auditory Verbal Learning Test (AVLT) and blood sampling were done before MRI scanning. Out of the 54 subjects, 28 with MoCA scores <26 were assigned to the MCI group whereas the 26 other participants were assigned to the control group with normal cognition (MoCA scores >26). Respective work durations were of 25.07±10.38 years for MCI participants and 27.88±5.03 years for the control group. Plasma aluminium levels were measured at 48.90±9.21 μg/L for patients and 32.51±6.05 μg/L for the control group, and there was no difference between the two groups.

After adjusting for confounding factors, the study observed an inverse relationship between plasma aluminium concentration and MoCA (r = -0.278; p = 0.036) and AVLT (-0.287; p = 0.035) scores. Cerebral MRI data were obtained for each participant and analysed using nonnegative matrix factorization. In MCI participants, the grey-mater volume of the default mode network (DMN) was lower than that in controls. Positive correlations were observed between DMN and MoCA scores, as well as between DMN and AVLT scores (Feifei Zhang et al. 2023).

Zhao D et al. (2023):

Zhao et al., investigated the relationship between plasma aluminium levels, lifestyle and cognitive function of 476 male workers from an electrolytic aluminium workshop and a repair workshop in an aluminium factory. Inclusion criteria were: age between 20 and 60 years and good physical condition. Exclusion criteria were: long-term use of aluminium-containing drugs, use of drugs affecting neurological functions during the past week, history of personal or familial neurological or mental disease, high frequency of cooking oil strips. Plasma aluminium concentration was measured by ICP-MS. Cognitive condition was determined using the MoCA. MCI was defined as a MoCA score

under 26. Subjects were 43.69±7.41 years old. Only 126 individuals had worked in aluminium industry for at least 10 years. Among them 49 were included in the MCI group and 77 considered as having normal cognitive performances. Despite this discrepancy, all 476 workers were categorized in four quartiles according to their plasma aluminium concentration, respectively Q1 <14.95 µg/l, Q2 14.95-32.96 µg/l, Q3 32.96-56.62 µg/l and Q4 >56.62 µg/l.

In this study, plasma aluminium concentration was associated with an increased risk of cognitive impairment for Q2, Q3 (not significant) and Q4 compared with Q1 participants. Derived OR (IC95%) were 2.102 (1.092-4.051), 1.866 (0.955-3.644) and 3.679 (1.928-7.020), respectively. The model was adjusted for income and marital status, education level, smoking status, drinking status, physical activity, daily reading time, daily mobile phone use, daily sleep duration (D. Zhao et al. 2023).

Meta-analysis:

A first meta-analysis was performed in 2007 by Meyer-Baron et al. It included the 9 studies by Hosovski et al (1990), Bast Pettersen et al. (1994 and 2000), Sjögren et al., 1996, Akila et al. (1999), Guo et al (1999), He et al 2003, Buchta et al 2003 and 2005. These nine studies globally concerned 449 exposed workers and 315 control subjects. The mean urinary aluminium concentrations in the exposed groups ranged from 13 to 133 µg/L. Six neuropsychological tests yielding 10 performance variables were analysed. A significant overall effect size was characterized for the digit symbol test (measuring speed-related components of cognitive and motor performances. The meta-analysis results also suggest an exposure-response relationship for this variable. This would constitute an argument for impairments of cognitive functions associated with occupational exposure to aluminium, even when urinary aluminium level is under 135 µg/L. However, the authors pertinently note that one significant effect size out of 10 analyses could be a chance result, and that uncertainties remain concerning the confounding factors that should and can be considered (Meyer-Baron et al. 2007).

A second meta-analysis was published in 2021 by Bagepally et al. It includes 23 studies, 1781 exposed and 1186 unexposed individuals. It shows statistically significant impairments of global cognitive scores, memory and working memory, associated with occupational aluminium exposure, but do not try to characterize a NOAEL or a LOAEL for these effects, using indicators of external exposure or biomarkers of exposure (Bagepally et al. 2021).

Vlasak et al., conducted a third meta-analysis of 18 studies of aluminium exposure association with performances in seven cognitive functions. It included, 1357 exposed and 1119 control individuals, however, the same longitudinal studies that are described in two separate papers are counted twice which might induce biased results. Results of the meta-analysis are reported using Hedges' g as aggregated effect size to consider small sample sizes of some integrated studies. Overall, exposed workers had impaired performances in the following cognitive outcomes: reaction time, working memory and processing speed. No association could be found between urine aluminium levels (corrected for creatinine or not) and cognitive performance while a linear association with blood aluminium levels is observed (Vlasak, Dujlovic, et Barth 2024).

4.8.1.1.1 Neurodegenerative diseases

Amyloid plaques and neurofibrillary tangles are significant neuropathological indicators of Alzheimer's disease. The presence of aluminium in senile plaques, primarily composed of aggregated β-amyloid peptides, and the occurrence of neurofibrillary tangles in the presence of aluminium offer some support for the link between Alzheimer's disease and aluminium presence in the brain (Bryliński et al. 2023). Considering the conflicting results of studies testing for an association between aluminium in brain and the risk of Alzheimer's disease, the simultaneous observation of these neuropathological features and aluminium in the brain does not establish the causal role of aluminium in Alzheimer's disease.

In a meta-analysis of three epidemiological studies (Virk et Eslick 2015), aluminium occupational exposure was not associated with Alzheimer's disease (OR, 1.00; 95% CI, 0.59 to 1.68). In another meta-analysis of eight epidemiological studies (Z. Wang et al. 2016), including also the three previous studies, an association of aluminium exposure with Alzheimer's disease was found (OR, 1.71; 95% CI, 1.35 to 2.18). When separating exposure through drinking water and occupational exposure, this association was observed only with exposure through drinking water (OR, 1.95; 95% CI, 1.47 to 2.59) while no association with occupational exposure was found (OR, 1.25; 95% CI, 0.80 to 1.94).

Most of the epidemiological studies testing for the association between aluminium concentration in drinking water and the risk of Alzheimer's disease and/or dementia suffer multiple methodological flaws. The main of these are that: 1) exposure to aluminium from water is generally not evaluated from individual repeated sampling of water really consumed by the participants, but from data issued from water distributing companies; they inconstantly take into accounts that the participants did not live at the same place during their whole life; 2) they generally only consider aluminium exposure through water, when it usually represents less than 5 % of the total exposure in the general population; 3) they generally do not take into account the other substances in drinking water (when positive and negative associations are respectively reported with the concentrations of fluorides or silicium in water and the risk of dementia). According to EFSA and WHO, exposure to aluminium through the food, including drinking water, does not constitute a risk for developing Alzheimer's disease (EFSA 2008; JECFA 2012).

4.8.1.1.2 Autism spectrum disorder

Autism spectrum disorder have initially been suggested to be associated with aluminium exposure through vaccines based on ecological studies. The Global Advisory Committee on Vaccine Safety (GACVS 2012) had assessed those studies. They presented limitations such as uncertainties regarding autism spectrum disorder prevalence in different countries or in vaccines schedule. More recently, a meta-analysis comprising 18 case-control studies with aluminium measurement in hair, blood and/or urine was published (Sulaiman, Wang, et Ren 2020). It shows equivocal associations between aluminium levels in biological matrices and autism spectrum disorder. While levels of aluminium in hair and urine were positively associated with autism spectrum disorder, aluminium levels in blood were negatively associated. Overall, these studies address association and cannot be used for causality assessment of the association of aluminium exposure with autism spectrum disorder.

Table VII.56: Epidemiological studies

Study	Population	Industry	Analytical method	BM	BM results in control group	BM results in workers	NOAEL / LOAEL	Results
Hosovski et al. (1990) Cross-sectional	Yugoslavia 87 Al-exposed workers 60 controls	Al foundry Al concentration in the workplace: 4.6 to 11.5 mg/m^3	Flameless AAS	Blood-Al	Mean: 58.09±74.73 µg/L	Mean: 136.85±103.15 µg/L	Blood-Al LOAEL: 136.85 µg/L U-Al LOAEL: 45.38 µg/L	Memory disorder, decreased performance in complex reaction tests, complicated manipulations and oculomotor coordination. No confounder considered except for alcohol and psychotropic drugs consumption within one month before the study. Probable massive external contamination of the blood samples
				U-Al	Mean: 7.25±7.82 µg/L.	Mean: 45.38±55.01 µg/L		
Bast-Pettersen et al. (1994) Cross-sectional	Norway 22 Al-exposed workers (potroom, foundry) 16 controls (other department)	Primary aluminium plant	Not reported	S-Al	Mean: 2.9 µg/L.	Mean: 3.6 µg/L in potroom workers Mean: 4.1 µg/L in foundry workers		Exposed workers reported more neuropsychiatric symptoms. Increased risk of impaired visuo-spatial organization in exposed workers but the differences are not significant with the control group
				U-Al	Mean: 7.8 µg/L	Mean: 12.6 µg/L in potroom workers Mean: 9.9 µg/L in foundry workers		
Sjögren et al. (1996) Iregren et al. (2001) Cross sectional	Sweden 173 Al exposed workers 39 mild steel welders	Al foundry and potroom workers, Al welders, workers in production of Al flake powder	GFAAS	Blood-Al	Median: 1.0 (range LOD-11) µg/L	Median: 1.0 (LOD-18), 9.0 (LOD-21) and 3.0 (LOD-27) µg/L in smelters, flake powder exposed workers and welders, respectively		No correlation between Al in urine and the outcomes measures. Groups not comparable.
				U-Al	Median: 4.7 µg/g creatinine	Median: 4.2, 59.0 and 24.0 µg/g creatinine for the smelters, flake powder exposed workers and welders, respectively		

Study	Population	Industry	Analytical method	BM	BM results in control group	BM results in workers	NOAEL / LOAEL	Results
Akila et al. (1999) Cross-sectional	Finland 51 aluminium welders 28 age-matched steel welders	Aluminium welders	GFAAS	S-Al	Not reported	Not reported		Effects of Al are only significant at high exposure group for Digit symbol substitution test, Item selection time, Block design, Backward counting.
				U-Al	<1 µmol/L (mean=0.46 µmol/L) < 27 µg/L (mean=12,4 µg/L)	Low exposure group: 1.1–4.0 µmol/L (mean=2.25 µmol/L); 27-108 µg/L (mean=60.7 µg/L) High exposure group: >4.1 µmol/L (mean=9.98 µmol/L); > 108 µg/L (mean=269.3 µg/L)		
Hänninen et al. (1994) ; Riihimäki et al. (2000) Cross-sectional	Finland 65 Al welders Control: 25 mild steel welders	Aluminium welders	GFAAS	S-Al	Median: 0.08 µmol/L (2.2 µg/L) Range: 0.04 to 0.12 µmol/L (1-3.2 µg/L)	Median (range): Low exposure group: 0.14 µmol/L (0.07-0.24 µmol/L); 3.8 µg/L (1.9-6.5 µg/L) High exposure group: 0.46 µmol/L (0.27-1.00 µmol/L); 12.4 µg/L (7.3-27 µg/L)		Exposition related impairments in cancellation accuracy (Bourdon-Wiesma test), backward counting, both components of dual task, synonyms, and memory for designs. Age-adjusted correlations between U-Al and impairments in Digit symbol test, backward counting, duela task cancellation speed and counting speed. No statistically significant differences between the groups in EEG quantitative analysis. No significant association was observed between serum or urine aluminium concentration and P300 amplitude or latency.
				U-Al	Median: 0,4 µmol/L (10.8 µg/L) Range: 0.1 to 1.3 µmol/L (2,7-35.1 µg/L)	Low exposure group: 1.8 µmol/L (0.3-5.7 µmol/L); 48.6 µg/L (8.1-153.9 µg/L) High exposure group: 7.1 µmol/L (3.2-27.3 µmol/L); 191.7 µg/L (86.4-737.1 µg/L)		

Study	Population	Industry	Analytical method	BM	BM results in control group	BM results in workers	NOAEL / LOAEL	Results
Bast-Pettersen et al. (2000) Cross-sectional	Norway 20 Al welders 20 construction workers = control	Aluminium welders	GFAAS	U-Al pre and post shift	Not measured	Median (range): 0.15 µmol/mmol creatinine (0.06-0.43 µmol/mmol creatinine); 35.8 µg/g creatinine; 14.3-109.9 µg/g creatinine)		Welders reported significantly more symptoms than controls and years of exposure were associated with poorer performance on the tremor test
Letzel et al. (2000) Longitudinal study	Germany 32 (E1) and 21 (E2) workers 30 (E1) and 15 (E2) controls	Al powder plant	Not reported	P-Al	Median: 1st examination: 4.3 µg/L 2nd examination: 4.3 µg/L	Median: 1st examination: 8.7 µg/L 2nd examination: 6.7 µg/L	NOAEL: 8.7 µg/L NOAEL: 87.6 µg/g creatinine	No significant exposure-related differences regarding performances in the psychometric tests or P300 potentials.
				U-Al	Median: 1st examination: 9.0 µg/g creatinine 2nd examination: 4.5 µg/g creatinine	Median: 1st examination: 87.6 µg/g creatinine 2nd examination: 19.8 µg/g creatinine		
Polizzi et al. (2002) Cross sectional study	Italy 64 al-exposed workers 32 controls All aprticipants retired for 10 years or more	Foundries	GFAAS	S-Al	Mean: 8.2 ± 1.17 µg/L	Mean: 14.1 ± 3.50 µg/L	LOAEL: 14.1 µg/L	Negative correlation between serum aluminium and the MMSE, MMSE-AE and CDT scores Positive correlation between serum aluminium and the MMSE and CDT times ERP-P300 latency positively correlated with serum aluminium concentration

Study	Population	Industry	Analytical method	BM	BM results in control group	BM results in workers	NOAEL / LOAEL	Results
Buchta et al. (2003) and Kiesswetter et al. (2009) Longitudinal study (4years)	Germany 98, 97, 92 Al welders 50 controls	Al welders of car-body construction industry	GFAAS	P-Al (post-shift)	Median (min, max): 1st: 4.4 (1.4-31.6) µg/L 2nd: 2.3 (0.7-5.9) µg/L 3rd: 3.8 (1.6-10.0) µg/L	Median (min, max): 1st: 8.3 (2.3–42.3) µg/L 2nd: 4.1 (0.72–11.7) µg/L 3rd: 4.3 (1.8-15.6) µg/L	P-Al NOAEL: 8.3 µg/L U-Al NOAEL: 37.87 µg/g creatinine	No differences in neurological symptoms report and in performances in psychometric tests, except that welders were slightly slower in their decision time but quicker in their motor movements.
				U-Al (post-shift)	Median (min, max): 1st: 5.2 (1.7-30.3) µg/g creatinine 2nd: 6.0 (1.6-390.0) µg/g creatinine 3rd: 5.0 (0.2-40.3) µg/g creatinine	Median (min, max): 1st: 37.87 (7.0–120.5) µg/g creatinine 2nd: 33.57 (9.0–230.11) µg/g creatinine 3rd: 15.4 (0.7-94.9) µg/g creatinine		
Buchta et al. (2005) and Kiesswetter et al. (2007) Longitudinal study (4years)	Germany 44 Al-welders 37 controls	5 companies in the train body and truck trailer construction industry	GFAAS	P-Al (post-shift)	Median: 1st: 3.5 µg/L 2nd: 2.8 µg/L 3rd: 4.5 µg/L	Median: 1st: 11.6 µg/L 2nd: 14.3 µg/L 3rd: 13.2 µg/L	P-Al LOAEL: 11.6 µg/L U-Al LOAEL: 97 µg/g creatinine	Observed significant difference between welders and controls only for block design test scores. Lowered performances (intergroup differences were not statistically significant) in welders group for verbal IQ, SPM test, trail making test, line tracing test and switching attention tasks.
				U-Al (post-shift)	Median: 1st: 5.8 µg/L or 4 µg/g creatinine 2nd: 6 µg/L or 4.5 µg/g creatinine	Median: 1st: 130 µg/L or 97 µg/g creatinine 2nd: 145.5 µg/L or 143.9 µg/g creatinine		

Study	Population	Industry	Analytical method	BM	BM results in control group	BM results in workers	NOAEL / LOAEL	Results
					3rd: 8.3 µg/L or 8.5 µg/g creatinine	3rd: 93.7 µg/L or 64.5 µg/g creatinine		
Giorgianni et al. (2014) Cross-sectional	Italy 86 Al-welders Control group (from the same plant): 90	Welders in a shipyard using MIG (metal inert gas) technique	Spectrophotometry technique of absorption fitted with a graphite oven	S-Al (Monday morning)	Mean: 6.93± 1.95 µg/L	Mean: 24.19±9.99 µg/L	LOAEL: 24.19 µg/L	Impaired scores of exposed workers in all cognitive tests, especially WMS test, Stroop test and attention matrices test
Mohammed et al. (2020) Cross-sectional	Egypt 75 Al exposed workers 75 controls from the same plant	Foundry (exposed to various metals)	Atomic Absorption Spectrometry (AAS)	S-Al	Mean ±SD: 0.36 mg/L ± 0.11	Mean ±SD: 0.56 mg/L ± 0.18	LOAEL: 560 µg/L	Significant decline in cognitive performance in the exposed group negatively correlated to S-Al and tau protein level. Probable massive external contamination of the blood samples
Guo et al. (1999) Cross-sectional	China 103 Al-exposed workers 64 controls	Large aluminium production plant (electrolysis, smelting or welding departments)	GFAAS	U-Al post-shift	Mean (range): 17.7 µg/g creatinine (3.5-42.8 µg/g creatinine)	Mean (range): 41.8 µg/g creatinine (14-9-116.2 µg/g creatinine)	LOAEL: 41.8 µg/g creatinine	Significant higher score of some negative mood variables of the POMS questionnaire in workers and inconsistent impairment in some psychometric tests (age dependent) in the group of workers

Study	Population	Industry	Analytical method	BM	BM results in control group	BM results in workers	NOAEL / LOAEL	Results
He et al. (2003) Cross-sectional	China 33 workers from a Chinese Al plant 34 controls from a flour mill.	Chinese aluminium plant	GFAAS	U-Al pre shift	Mean: 26.8 µg/g creatinine	Mean: 40.1 µg/g creatinine	LOAEL: 40.1 µg/g creatinine	Reaction time was significantly slower for exposed workers and scores of the digital symbol test and pursuit aiming test were significantly lower in this group.
Yang et al. (2015) Cross-sectional	China 366 Al potroom workers	Al potroom	GFAAS	S-Al	-	Median (range): 48.99 µg/L (6.63-158.8 µg/L)		Total MMSE score decrease with S-Al increase Risk of mild cognitive impairment (MCI) increase with S-Al increase
Meng et al. (2019) Cross-sectional	China 853 Al-exposed workers from which only 334 were fully evaluated 1336 matched controls	Al factory	ICP-MS	P-Al	Median (P25-P75): (12.02 (6.35, 20.86) µg/L)	Median (P25-P75) in the 53 MCI patients: 18.17 (10.39, 34.96) µg/L)		High P-Al increased the risk of cognitive problems, advanced education was a protective factor
Wang et al. (2020) cross-sectional	China 831 Al-exposed workers	Al factory	ICP-MS	P-Al	-	Four quartiles: Q1:0-8.28 µg/L Q2: 8.28-15.26 µg/L Q3: 15.26-27.02 µg/L Q4: ≥27.02 µg/L		Positive association between P-Al and cognitive impairments

Study	Population	Industry	Analytical method	BM	BM results in control group	BM results in workers	NOAEL / LOAEL	Results
Lu et al. (2021) Longitudinal study (2 years)	China 276 Al-exposed workers	Al factory	ICP-MS	P-Al	-	3 tertiles: T1: <17.6 µg/L T2: 17.6-37.3 µg/L T3: ≥37.3 µg/L)		Observed dose-response negative relationship between P-Al and MMSE score and FOME score
Xu et al. (2021) Cross-sectional study	China 1660 exposed workers	Al plant	ICP-MS	P-Al	-	Median: 34.5 (P25, P75 =15.0, 42.3) µg/L		P-Al negatively correlated with DST and DSBT test scores.
Zhang et al. (2021) Cross-sectional	China 539 Al-exposed workers 1720 unexposed	Aluminium niners and workers from remated factories	No measurement	-	-	-		Exposed people had a significant lower MMSE score ($p<0.001$) and a higher risk of cognitive impairment (OR 2.21)
Shang et al. (2021) Cross-sectional	China 187 aluminium workers	Aluminium factory	ICP-MS	P-Al	-	Median (IQR): Normal group: 55.862 (38.701–77.012) µg/L MCI group: 72.794 (42.510–102.652) µg/L for the MCI group.		Significant inverse relationship between P-Al and MocA total scores
Zhang Z et al. (2022) Cross-sectional	China 53 Al-exposed workers	Al factory	ICP-MS	P-Al	-	Mean level: Normal group: 33.1 µg/L MCI group: 43.8 µg/L		28 workers had mild MCI P-Al was higher in workers with MCI QSM exploration showed higher values in several brain regions for the MCI group No correlation between P-Al and QSM values.

Study	Population	Industry	Analytical method	BM	BM results in control group	BM results in workers	NOAEL / LOAEL	Results
Zhao et al. (2022) Cross-sectional	China 352 al-exposed workers	Al factory	GFAAS	P-Al	-	Four quartiles: Q1 <17.7 µg/l; Q2 17.7-27.85 µg/l; Q3 27.85-41.04 µg/l; Q4 ≥ 41.04 µg/l		Association between P-Al increase and decrease of global score of cognitive impairment
Zhang Y et al. (2022) Cross-sectional	China 392 Al-exposed workers	Al factory	ICP-MS	P-Al	-	Four groups based on median and quartiles of plasma Al levels: Q1 (< 18.08 µg/L), Q2 (18.08-28.21 µg/L), Q3 (28.21-40.88 µg/L), Q4 (≥ 40.88 µg/L)		Decrease of MMSE, VFT and FOM scores with P-Al increase
Zhang et al. (2023) Case-control	China 28 Al- exposed workers with MCI 26 controls	Al factory	Not reported	P-Al	32.51±6.05 µg/L	48.90±9.21 µg/L for patients with MCI		Inverse relationship between P-Al and MocA and ALVL scores Lower gray matter volume in MCI patients at cerebral MRI examination
Zhao et al. (2023) Cross-sectional	China 476 Al-exposed workers	Al factory	ICP-MS	P-Al	-	Four quartiles: Q1 <14.95 µg/l, Q2 14.95-32.96 µg/l, Q3 32.96-56.62 µg/l and Q4 >56.62 µg/l		P-Al increase associated with increased risk of cognitive impairment

Urinary aluminium levels reported in the various studies with NOAELs or LOAELs are presented in the histogram below (Figure VII.8) along with the number of samples (the study by Hosovski et al. 1990 is excluded as it suffers severe flaws in exposure evaluation and urinary aluminium levels are not expressed in µg/g creatinine).

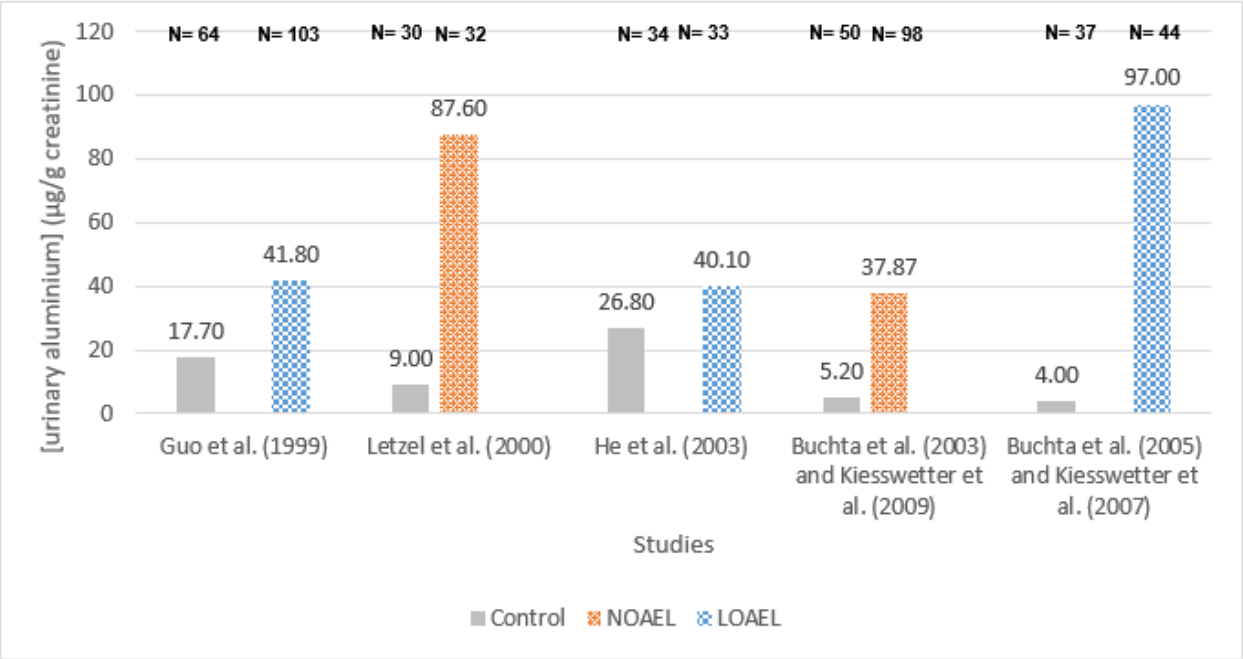

Figure VII.8: NOAEL and LOAEL extrapolated from median urinary concentrations measured in exposed workers in relation to cognitive impairment.

4.8.1.2 Animal data

No neurotoxicity studies have been found in animals following chronic exposure to aluminium compounds.

4.8.2 Respiratory toxicity

4.8.2.1 Human data

Numerous studies have documented respiratory effects linked to occupational exposure to aluminium, with diverse activities implicated such as aluminium smelting, electrolytic refineries, aluminium powder production, and aluminium welding. The spectrum of respiratory disorders includes wheezing, dyspnoea, impaired lung function, asthma, and pulmonary fibrosis.

However, the attribution of these disorders to aluminium exposure remains uncertain or even improbable in many studies due to confounding factors, particularly co-exposures to other toxic chemicals, especially irritating agents. For instance, co-exposure to hydrogen fluoride and other fluorides were reported in electrolytic refineries in cases of potroom asthma or pulmonary fibrosis; co-exposure to ozone and ultra-fine particles in workers exposed to welding fumes; co-exposure to crystalline silica in cases of fibrosis in workers exposed to bauxite (Krewski et al. 2007; ATSDR 2008). Conflicting data are reported concerning the pulmonary effects of finely ground aluminium powder: some publications report on cases of pulmonary fibrosis in exposed workers, when other studies show no evidence of fibrosis after prolonged exposure to aluminium fine particles. It is believed that these differences could be explained by the type of lubricant used to prevent surface oxidation of aluminium particles during milling. Stearic acid is the most commonly used lubricant; it reacts with aluminium forming a protective superficial film of aluminium stearate; no fibrogenic effect is reported when using this process. In contrast, the previous and now discontinued use of mineral oil, as a lubricant for aluminium milling has been associated with pulmonary fibrosis. Sporadic cases of pneumoconiosis associated with aluminium occupational exposure are also reported (Korogiannos, Babatsikou, et Tzimas 1998; Kraus et al. 2000; Hull et Abraham 2002). Their small number and the co-exposures to other chemical agents limit their interpretation.

4.8.2.2 Animal data

Limited data are available on the respiratory effects of aluminium and its compound following chronic inhalation (ATSDR 2008). Identified studies are described below and summarised in Table VII.57.

- **Mice**

No organ weight or histological changes were observed in the lungs of Swiss mice exposed over lifetime (2-2.5 years) to 1.2 mg Al/kg bw per day as aluminium sulphate in drinking water (Schroeder et Mitchener 1975b) or in mice exposed to 979 mg Al/kg bw per day as aluminium potassium sulphate in the feed for 20 months (Oneda et al. 1994).

- **Rats**

No organ weight or histological changes of the lungs were observed in Long- Evans rats exposed over 2 years to 0.6 mg Al/kg bw per day as aluminium sulphate in drinking water (Schroeder et Mitchener 1975a).

Rats (Fischer- 344) exposed over 12-24 months (5d/wk and 6hr/d) to aluminium chlorohydrate at doses of 0.25, 2.5 or 25 mg/m³ presented a 108-274% increase in relative lung weight at 2 years at the high exposure dose (LOAEL= 25 mg/m³ aluminium chlorohydrate, corresponding to 6.5 mg Al/m³). No effects on lungs weight were observed at the dose of 2.5 mg/m³ (NOAEL corresponding to 0.65 mg Al/m³) (Stone et al. 1979).
In a study by Pigott et al., no lung fibrosis was observed in rats (Wistar) following exposure to 2.18 or 2.45 mg/m³ alumina fibres (Saffil fibres or Saffil aged fibbers, median diameter ranging between 3.0-3.3 µm) over 86 weeks (5 d/wk and 6hr/d) (Pigott, Gaskell, et Ishmael 1981).

- **Guinea pigs**

Guinea Pigs (Hartley) exposed to aluminium chlorohydrate at doses of 0.25, 2.5 or 25 mg/m³ over 12-21 months (5d/wk and 6hr/d) showed a 21% increase in relative lung weight at 2 years at the lowest exposure dose (LOAEL corresponding to 0.065 mg Al/m³) (Stone et al. 1979).

Table VII.57: Animal studies on aluminium chronic exposure respiratory toxicity

Strain	Aluminium compound	Duration and route of exposure	Doses	Endpoint	NOAEL	LOAEL	Reference
Mice							
Swiss (n= 54 per group)	Aluminium sulphate	Lifetime Water	0 or 5 mg/l	Weight and histological changes in lungs	1.2 mg Al/kg bw per day		Schroeder and Mitchener (1975b)
B6C3F1 (n= 60 per group)	Aluminium potassium sulphate	20 months Feed	0, 1, 2.5, 5, 10% APS (w/w)	Weight and histological changes in lungs	979 mg Al/kg bw per day		Oneda et al. (1994)
Rats							
Long- Evans (n=52 of each sex per group)	Aluminium sulphate	2 years Water	0 or 5 mg/l	Weight and histological changes in lungs	0.6 mg Al/kg bw per day		Schroeder and Mitchener (1975a)
Fischer- 344 (n≈ 17 per group)	Aluminium chlorohydrate	12-24 months Inhalation	0, 0.25, 2.5 or 25 mg/m³	Increase in relative lung weight at 2 years	0.65 mg Al/m³	6.5 mg Al/m³	Stone et al. (1979)
Wistar (n= 50 per group)	Alumina fibres (Aluminium oxide)	86 weeks Inhalation	0, 2.18 or 2.45 mg/m³	Lung fibrosis	2.45 mg/m³		Pigott et al. (1981)

Strain	Aluminium compound	Duration and route of exposure	Doses	Endpoint	NOAEL	LOAEL	Reference
Guinea pigs							
Hartley (n= 15 per group)	Aluminium chlorohydrate	12-21 months Inhalation	0, 0.25, 2.5 or 25 mg/m³	Increase in relative lung weight at 2 years		0.065 mg Al/m³	Stone et al. (1979)

4.8.3 Haematological effects

4.8.3.1 Human data

No human studies were identified regarding haematological effects following chronic exposure to aluminium compounds. However, hypochromic and microcytic anaemia, unresponsive to iron therapy, have been reported in patients with chronic renal failure undergoing dialysis. This may be the result of interference with haem synthesis and/or increased haem oxygenase activity (Yokel et Sjögren 2022).

4.8.3.2 Animal data

In the study by Stone et al., rats (Fischer-344) and guinea pigs exposed to 0.25, 2.5 or 25 mg/m³ of aluminium chlorohydrate over 12-24 and 12-21 months respectively did not present haematological effects. The NOAEL of 25 mg/m³ Al chlorohydrate corresponds to of 5.4 mg Al/m³ (Stone et al. 1979).

4.8.4 Bone related effects

4.8.4.1 Human data

Osteomalacia has been reported in patients chronically taking aluminium-containing antacids, even in the absence of renal failure, due to phosphate imbalance. Indeed, a decrease in phosphate absorption has been observed after taking aluminium-containing antacids. In addition, fractures and bone pain have been reported in kidney failure patients on dialysis, suffering from osteomalacia, as well as in patients suffering from osteomalacia due to total parenteral nutrition. However, workers at an aluminium powder plant showed no difference in bone mineral density and bone mineral content of the lumbar spine compared with the control group, even though the workers had higher levels of urinary aluminium (Yokel et Sjögren 2022).

4.8.4.2 Animal data

No animal studies were identified regarding musculoskeletal effects following chronic inhalation or chronic oral exposure to aluminium compounds.

4.8.5 Other effects

4.8.5.1 Human data

No reliable human studies in the general population or workers on systemic toxicity were identified following chronic exposure to aluminium compounds.

A slightly higher mortality rate from cardiovascular disease has been reported among miners exposed to aluminium. However, the pathologies observed in miners are difficult to interpret, as the dust is not made up of aluminium alone, but of other pathogenic elements, notably crystalline silica, and as other non-chemical risk factors of cardiovascular disease are associated with mining activity, especially strong muscular activity in a hot and wet environment.

4.8.5.2 Animal data

Studies reporting systemic effects following chronic exposure to aluminium and its compounds were documented in the ATSDR (2008) report. The studies are described below and summarised in Table VII.58.

- **Mice**

Swiss mice exposed over lifetime to aluminium sulphate via water (5 ppm aluminium) did not show histological changes in the heart, kidney or liver and no effects were observed on the body and organs weights (NOAEL of 1.2 mg Al/kg bw per day) (Schroeder et Mitchener 1975b).

Swiss-Webster mice were exposed to aluminium lactate for 2 years (from conception to month 24) at 7 (control) or 100 µg Al/g diet. A 20% decrease in body weight gain was reported in females at the dose of 100 mg Al/kg bw per day as aluminium lactate (LOAEL). However, alterations in body weights were not observed in another group of mice similarly exposed (Golub et al. 2000).

- **Rats**

Rats (Long- Evans) exposed for 2.5 years to aluminium sulphate via water (5 ppm aluminium) did not show histological changes in the heart, kidney or liver nor effects on the body weight (NOAEL of 0.6 mg Al/kg bw per day) (Schroeder et Mitchener 1975a).

Fischer-344 rats exposed to the high dose of aluminium chlorohydrate (0, 0.25, 2.5 and 25 mg/m^3) had a significant decrease in their body weight at 12 and 24 months of exposure (LOAEL of 25 mg/m^3 aluminium chlorohydrate, equivalent to 6.5 mg Al/m^3 and NOAEL of 2.5 mg/m^3 aluminium chlorohydrate equivalent to 0.65 mg Al/m^3) (Stone et al. 1979).

Finally, Sprague-Dawley rats exposed from conception to sacrifice (at 1 year or 2 years) to 0, 50 or 100 mg Al/kg/day as aluminium nitrate through water (+ citric acid to increase aluminium absorption), did not show any adverse body weight effect (Roig et al. 2006). The NOAEL was of 100 mg Al/ bw per day.

- **Guinea pigs**

No effect on the body weight was observed in guinea pigs exposed to 0.25, 2.5 or 25 mg/m^3 of aluminium chlorohydrate over 12-21 months (NOAEL = 25 mg/m^3 aluminium chlorohydrate , equivalent to 6.5 mg Al/m^3) (Stone et al. 1979).

Table VII.58: Animal studies on aluminium chronic exposure systemic toxicity

Strain	Aluminium compound	Duration and route of exposure	Doses	Endpoint	NOAEL	LOAEL	Reference
Mice							
Swiss (n= 54 per group)	Aluminium sulphate	Lifetime Water	0 or 5 mg/l	Histological changes in heart, kidney, liver and the body weight	1.2 mg Al/kg bw per day		Schroeder and Mitchener (1975b)
Swiss-Webster (n= 18 per group)	Aluminium lactate	Conception-24 months Diet	7 (control) or 100 µg Al/g diet	20% decrease in body weight gain in female mice		100 mg Al/kg bw per day	Golub et al. (2000)
Rats							
Long-Evans (n=52 of each sex per group)	Aluminium sulphate	2 years Water	0 or 5 mg/l	Histological changes in heart, kidney, liver and the body weight	0.6 mg Al/kg bw per day		Schroeder and Mitchener (1975a)
Fischer-344	Aluminium chlorohydrate	12-24 months Inhalation	0, 0.25, 2.5 or 25 mg/m^3	Decrease in the body weight	0.65 mg Al/m^3	6.5 mg Al/m^3	Stone et al. (1979)

Strain	Aluminium compound	Duration and route of exposure	Doses	Endpoint	NOAEL	LOAEL	Reference
(n≈ 17 per group)							
Sprague-Dawley (n= 15-21 per group)	Aluminium nitrate + citric acid	Conception- 1 or 2 years olds Water	0, 50 or 100 mg Al/kg/day	No effect on the body weight at the highest tested dose	100 mg Al/ bw per day		Roig et al. (2006)
Guinea pigs							
Hartley (n= 15 per group)	Aluminium chlorohydrate	12-21 months Inhalation	0, 0.25, 2.5 or 25 mg/m^3	No effect on the body weight at the highest tested dose	6.5 mg Al/m^3		Stone et al. (1979)

4.8.6 Toxicity on reproduction and developmental toxicity

4.8.6.1 Human data

No reliable studies have been identified regarding the reproductive effects and developmental toxicity of aluminium or its compounds in humans.

4.8.6.2 Animal data

ATSDR (2008) and EFSA (2008) reported some studies regarding reproductive effects and developmental toxicity of aluminium compounds in animals. Identified studies are described below and summarised in TableTable VII.59,

Table VII.60 and Table VII.61.

- **Mice**

An increased incidence of resorptions was reported in female BALB/c mice treated with 200 or 300 mg/kg bw per day of aluminium chloride, through gavage, on Gds 7-16 (Cranmer et al. 1986). The control group received a saline solution intraperitoneally (ip). The LOAEL for increased resorption is of 200 mg/kg bw per day $AlCl_3$ equivalent to 41 mg Al/kg bw per day.

In female Swiss-Webster mice exposed through the diet to aluminium lactate (25 (control), 500 or 1000 µg Al/g diet), during gestation and lactation (Gd1-Ld21), an altered gestational length was observed in the 500 and 1000 µg Al/g groups, where some pups were born on Gd 17, 19 or 20 whereas all pups were born on Gd18 in the control group. The LOAEL for reproductive effects was equivalent to 155 mg Al/kg bw per day. Furthermore, no effects were observed on the pregnancy rate, litter size, birth weight, peri and post-natal pup mortality, even in the group with the highest exposure approximatively equivalent to 310 mg Al/kg bw per day (NOAEL for developmental toxicity). In this study, mice fed with aluminium lactate from conception through weaning had a decrease in forelimb strength (age of 39 days), an increase in hind limb grip strength (age of 25 days) and an increase in foot splay in weanling (age of 21 and 35 days) at the dose of 500 µg Al/g diet (Donald et al. 1989). The LOAEL for neurodevelopmental toxicity was of 500 µg Al/g diet, corresponding to 155 mg Al/kg bw per day.

Pregnant Swiss mice exposed through gavage during Gd 6 to 15 to aluminium hydroxide at doses of 0, 66.5, 133 or 266 mg/kg bw per day corresponding to 0, 23, 46 or 92 mg Al/kg bw per day did not have maternal toxicity or signs of embryotoxicity including morphological abnormalities (Domingo et al. 1989). The NOAEL was thus reported to be 92 mg Al/ kg bw per day.

In a study by Golub et al., Swiss-Webster mice were exposed to aluminium lactate at doses of 25 (control) or 1000 µg Al/g diet, through the diet, from conception through gestation, or from conception to lactation, or during lactation only (Golub, Keen, et Gershwin 1992a). There was a significant decrease in pup body weight, crown-rump length and forelimb grip strength in the gestation exposed group. There was an increase in hind limb grip strength and in tail withdrawal times in gestation and lactation exposed groups. An increase in negative geotaxis latency was observed in the lactation exposed groups. The LOAEL was of 1000 µg/g diet, equivalent to 250 mg Al/kg bw per day. In this study, no reproductive effects (no effects on litter size, birth weight, crown-rump length, or sex ratio) were seen in female mice dams exposed to aluminium lactate during gestation and lactation. The NOAEL for reproductive toxicity is equivalent to 250 mg Al/kg bw per day.

In another study, Swiss (CD-1) mice were exposed during Gd 6 to 15, by gavage, to either aluminium lactate (627 mg/kg bw delivering 57.4 mg Al/kg bw per day) or aluminium hydroxide (166 mg/kg bw delivering 57.4 mg Al/kg bw per day) or aluminium hydroxide and lactic acid (570 mg/kg bw) or lactic acid or distilled water. It was shown that dams had a reduced body weight gain (not related to food consumption) following exposure to either aluminium lactate or aluminium hydroxide + lactic acid. In addition, exposure to aluminium lactate induced a significant decrease of foetal body weight, cleft palate and delayed foetal ossification (M. T. Colomina et al. 1992). The LOAEL for aluminium lactate was of 57.4 mg Al/Kg bw per day and the NOAEL for aluminium hydroxide of 57.4 mg Al/kg bw per day.

Aluminium hydroxide (dose of 300 mg/kg bw per day delivering 103 mg Al/kg bw per day) was also administered to Swiss mice, by gavage, with or without ascorbic acid (85 mg/kg bw per day) from Gd 6 to Gd 15. The study included a control group receiving distilled water. A significant reduction in maternal food consumption was reported during all the gestational period in the aluminium hydroxide and aluminium hydroxide + ascorbic acid groups. No developmental effects were observed among the groups including the percentage of post implantation loss, foetal body weight, and the incidence of minor anomalies and major malformations (M. T. Colomina et al. 1994). The NOAEL was of 103 mg Al/kg bw per day.

Swiss-Webster mice were exposed to aluminium lactate in the diet at 7 (control), 500 or 1000 µg Al/g diet from conception until weaning. At 500 µg Al/g diet, there was a decrease in forelimb and hind limb grip strengths and startle response compared to controls (Golub et al. 1995). The LOAEL was of 500 µg/g diet, that is 155 mg Al/kg bw per day.

In a study by Golub and Germann, where Swiss-Webster mice were exposed through diet, from conception till PND 35 to aluminium lactate at doses of 7 (control), 500 or 1000 µg Al/g diet, there was no effect of aluminium exposure on the performance of the Delayed Spatial Alternation task (Golub et Germann 1998). The NOAEL was of 1000 µg/g diet, that is 330 mg Al/kg bw per day.

In a study where five groups of CD-1 mice were exposed by gavage to aluminium nitrate nonahydrate at a single dose of 995 mg/kg bw (71 mg Al/kg bw), at one of the Gds 8 to 12, a reduction of body weight gain and a reduced foetal body weight were observed in all aluminium treated groups compared to controls. In addition, reduced ossification was common due to the exposure to aluminium (in all groups compared to controls). Some female death and abortions were also reported (Albina et al. 2000). The LOAEL was of 71mg Al/kg bw in this study.

Furthermore, a chronic exposure to aluminium lactate through the diet (at 7 (control) or 1000 µg Al/g diet) over 2 years, from conception until month 24 of age caused a decrease in forelimb and hind limb strength and a decreased thermal activity in aluminium-treated Swiss-Webster mice (Golub et al. 2000). The LOAEL was of is of 1000 µg/g diet equivalent to 100 mg Al/kg bw per day.

Swiss Webster mice were exposed to aluminium lactate through the diet at doses of 7 (control), 100, 500, or 1000 µg Al/g diet from conception to PND 35. From the dose of 500 µg Al/g diet, females showed an impaired performance on the water maze test and males had shorter latency to fall in wire suspension test. At 1000 µg Al/g diet there was a decrease in hind limb grip strength compared to controls (Golub et Germann 2001). The LOAEL was of 500 µg/g diet that is 130 mg Al/kg bw per day and the NOAEL was of 100 µg/g diet, that is 26 mg Al/kg bw per day.

Pregnant Swiss-Webster mice were exposed to 0, 300 or 600 mg Al/kg bw per day as aluminium chloride through water from Gd 1 until PND 15. For each experimental group, pups were culled to 8 per dam but stayed with their mothers until PND 22. In male offspring, a significant and dose-dependent deficit was reported in the locomotor activity (PND 22), learning capacity (PND 25) and cognitive behaviours (PND 30-36). In addition, delays in opening of the eyes and appearance of body hair fuzz, and deficits in the sensory motor reflexes of the pups during weaning period were reported (Abu-Taweel, Ajarem, et Ahmad 2012). The LOAEL was of 300 mg Al/kg bw per day.

- **Rats**

In the study by Steinhagen et al., Fischer-344 rats inhaling 0, 0.065, 0.65 or 6.5 mg Al/m^3 as aluminium chlorohydrate over 6 months had no histological changes in their reproductive tissues (Steinhagen, Cavender, et Cockrell 1978). The NOAEL is of 6.5 mg Al/m^3.

Aluminium chloride was given to Wistar rats, through diet, at doses of 0, 160 or 200 mg Al/kg bw per day, from Gd 8 until parturition. There was no difference between the aluminium treated groups and the control group regarding food consumption and weight gain of pregnant rats. Aluminium affected postnatal pups' survival but not in a dose dependent manner. The age of appearance of eye opening and mean body weight of pups did not differ between groups except for the body weight that was reduced on the first day postpartum in the treated groups compared to controls (V. Bernuzzi, Desor, et Lehr 1986). The LOAEL was of 160 mg Al/kg bw per day.

In a study by Domingo et al., male Sprague-Dawley rats were exposed to aluminium nitrate nonahydrate through gavage, over 60 days prior to mating, at the following doses: 0, 180, 360 or 720 mg/kg bw per day. Female rates were exposed to similar doses for 14 days prior to mating (with male rat with the same exposure dose), during gestation, delivery and lactation. No reproductive toxicity was observed in male and female rats (Domingo et al. 1987a). NOAEL regarding male and female fertility was considered to be of 720 mg/kg bw per day of aluminium nitrate nonahydrate, equal to 52 mg Al/ kg bw per day.

Domingo et al., administered aluminium nitrate by gavage at doses of 0, 180, 360 or 720 mg/kg bw per day (delivering 0, 13, 26 or 52 mg Al/kg bw per day) to pregnant Sprague Dawley rats (10 per group) from Gd14 to Gd21. Number of litters and number of live pups per litter were lower in all aluminium treated groups compared to controls. However, the decrease was not significantly dose dependent. At the highest dose of aluminium nitrate, the mean pup body weight was lower (Domingo et al. 1987c). The LOAEL was of 13 mg Al/kg bw per day.

In another study, aluminium nitrate was administered to rats (Sprague Dawley, 10 per group), by gavage, from gestational day 6 to gestational day 14, at doses of 0, 180, 360 or 720 mg/kg bw per day (delivering 0, 13, 26 or 52 mg Al/kg bw per day), then, rats underwent caesarean section on Gd20. Results showed that all groups exposed to

aluminium nitrate had a significantly reduced body weight gain of dams compared to controls. The number of runt foetuses was higher, in an aluminium dose dependent manner. In addition, foetal body weight was reduced in all the aluminium treated group where severe signs of delayed ossification, increase congenital malformations and minor anomalies were observed (Paternain et al. 1988). The LOAEL was 13 mg Al/kg bw per day.

Bernuzzi et al., administered aluminium chloride (0, 100, 300 or 400 mg/kg bw per day delivering 0, 20, 60 and 80mg Al/kg bw per day) or aluminium lactate (0, 100, 300 or 400 mg/kg bw per day delivering 0, 9, 18 and 36 mg Al/kg bw per day), through diet, to pregnant Wistar rats from Gd 1 to parturition. In the mid and high doses of aluminium chloride and in the high dose of aluminium lactate, a reduction of maternal body weight gain, higher postnatal mortalities and significant reduction of pup weights at birth and during postnatal development were observed compared to controls. Litter size at birth was similar in all the groups (Viviane Bernuzzi, Desor, et Lehr 1989a). The NOAEL was of 20 mg Al/kg bw per day in rats treated with aluminium chloride and the of 18 mg Al/kg bw per day for those treated with aluminium lactate. The LOAEL were 60 and 36 mg Al/kg bw, respectively.

In another study, aluminium lactate was administered through diet at 0 or 400 mg Al/kg bw per day to pregnant Wistar rats during Gd1-Gd7 or Gd1-Gd14 or Gd1-partution. The results showed no effect of aluminium on the litter size, mortality rate or weight of pups. Maternal body weight was significantly decreased on Gd 16 and Gd 19 in the group of rats treated during the whole gestational period (Muller et al. 1990). The NOAEL for developmental effects was of 400 mg Al/kg bw per day.

Wistar rats were administered, by gavage, aluminium chloride on days 6 to 15 of gestation at doses delivering 0, 66, 132 or 264 mg Al/kg bw per day (divided in two equal administrations per day). Dams were killed on day 20 of gestation. No maternal toxicity nor embryotoxicity were reported in all groups (Gomez et al. 1990). The NOAEL was 264 mg Al/kg bw per day.

Female rats (Sprague-Dawley) were exposed for 15 days prior to mating, then during gestation, lactation and post-weaning period to 0, 50 or 100 mg Al/kg bw per day as aluminium nitrate nanohydrate in drinking water (citric acid was added at 335 or 710 mg/kg/day) (M. Teresa Colomina et al. 2005). At 100 mg Al/kg bw per day, a decrease in the body weight of pups was observed from postnatal day 12 through 21. A delay in vaginal opening was reported from the dose of 50 mg Al/kg bw per day and a delay in testes descent was reported from 100 mg Al/kg bw per day. The LOAEL for developmental toxicity was of 50 mg Al/kg bw per day. In this study, a decrease in forelimb strength of pups was observed at 100 mg Al/kg bw per day. The LOAEL for neurodevelopmental effects was of 100 mg Al/kg bw per day for these effects and the NOAEL of 50 mg Al/kg bw per day.

However, Sprague Dawley rats did not have alterations in performance of water maze test when exposed to 100 mg Al/kg bw per day as aluminium lactate (NOAEL) from conception to sacrifice (at 1 year or 2 years old) (Roig et al. 2006). In this study, citric acid was added to the water and aluminium was administered at doses of 0, 50 or 100 mg Al/kg bw per day.

In the study by Erazi et al., Wistar rats were exposed to aluminium chloride during gestational and lactation stages and then after weaning in their drinking water (0 or 3 g/l $AlCl_3$) until the age of 4 months. There was a significant increase of glial fibrillary acidic protein (GFA-P) -immunoreactive astrocytes in brain of aluminium treated rats who also had a significant reduced locomotor activity compared to controls. Also, rats exposed to aluminium preferred to spend more time in the lit compartment of a dark/light box, which indicates increased anxiety; this was not seen in rats only exposed to aluminium at adult age in this same study (Erazi et al. 2010). The LOAEL was of 3000 mg/ aluminium chloride per liter, that is 600 mg Al/L.

In a study conducted according to GLP with a design based on OECD Test Guideline 426, Sprague Dawley pregnant rats were exposed to aluminium citrate through drinking water, starting gestational day 6, during gestation, lactation, and to offspring during post-weaning, through to PND 364, at doses delivering 30, 100 or 300 mg Al/kg bw per day. The study also included two control groups receiving either sodium citrate solution or deionized water. The concentration of aluminium in the diet was reported to be less than 9 µg/kg. On PND 4, there was a normalisation to 4 males and 4 females per litter assigned per number to each of four sacrifice day groups (day 23, day 64, day 120, day 364) associated with milestone observations and sacrifice. In the offspring, white precipitates were observed in the urinary tracts (test item precipitation) resulting in hydronephrosis, ureteral dilation and stone formation, this effect was considered related to aluminium-treatment and was most prominently observed in the high dose group, particularly in male pups, which resulted in higher mortality and morbidity rate in male

pups of the high dose group. The high dose group was considered to be the maximum tolerated dose. In the middle dose group, urinary tract lesions, decreased body weight in males at PND 120, elevated fluid consumption and, an exaggerated response to tail pinch and narrower foot splay in females. There was also a decrease in hindlimb and forelimb grip strength in pups (significant in the mid- and high dose) that was dose related, although some of the effects may be secondary to body weight changes. No significant effects were reported for auditory startle response, T-maze tests or the Morris water maze test. The authors concluded a LOAEL of 100 mg Al/kg bw per day and a NOAEL of 30 mg Al/kg bw per day (Poirier et al. 2011).

Sun and al., conducted a study on male Wistar rats, exposed orally, for 120 days, to 0, 64.18, 128.36 or 256.72 mg/kg bw per day of aluminium chloride in drinking water. Aluminium treated male rats had decreased levels of testosterone and luteinizing hormone (LH) when exposed to ≥ 128.36 mg/kg bw per day compared to controls; in all aluminium treated groups follicle-stimulating hormone (FSH) level did not show significant change compared to controls however, androgen receptor protein expression and mRNA expression were lower than in control group (H. Sun et al. 2011). The LOAEL was of 64.18 mg/kg bw per day of aluminium chloride, equivalent to 13 mg Al/kg bw per day.

In a study by Wang et al., female Wistar rats were administered 0, 64.18, 128.36, or 256.72 mg /kg bw per day of aluminium chloride through their drinking water over 120 days. It was shown that aluminium exposed female rats, in the three aluminium exposure groups, had a significant decrease in serum levels of oestrogens, progesterone, FSH and LH in an aluminium dose-dependent manner, compared to the control group (N. Wang et al. 2012). The LOAEL was 64.18 mg/kg bw per day of aluminium chloride, equivalent to 13 mg Al/kg bw per day.

Pregnant Wistar rats were divided into four groups: from the first day after birth, they received water containing 0, 0.2, 0.4 or 0.6% of aluminium chloride. Eight litters per group were kept and were exposed to aluminium chloride through lactation from parturition to weaning (3 weeks) and then were administered water containing 0, 0.2, 0.4, 0.6% of aluminium chloride till 3 months age. Pups exposed to aluminium chloride had higher levels of aluminium in blood and hippocampus than controls and, aluminium caused pathological changes in neuronal and synaptic ultrastructure and impaired spatial memory ability in rats (C. Zhang et al. 2013). The LOAEL was of 0.2 % aluminium chloride in drinking water, that is 400 mg Al/L.

Wistar male rats were orally exposed to 0, 64.18, 128.36 or 256.72mg/kg bw per day of aluminium chloride for 120 days. Findings of this study (Zhu et al. 2014) showed that aluminium caused adverse effects on testicular function; exposed rats had a decrease in Zn and Fe testes content (mid and high dose), sperm count (mid and high dose) and enzyme activities of testicular ACP (acid phosphatase), LDH-x (lactate dehydrogenase isoenzyme) (mid and high dose), SDH (succinate dehydrogenase) and LDH (lactate dehydrogenase) (high dose) with an increase in aluminium and Cu contents (mid and high dose) and an increase in sperm malformation rate (in all the aluminium-treated groups, 31.24% in the highest exposed group vs 14.93% in the control group). LOAEL was of 64.18 mg/kg bw per day of aluminium chloride, equivalent to 13 mg Al/kg bw per day.

In a more recent study, female Wistar rats were exposed to 0, 64, 128 or 256 mg/kg bw per day of aluminium chloride in drinking water over 120 days. Results showed that $AlCl_3$-treated rats had a disruption in their ovary structure (at high dose), a decrease in the activities of ALP, ACP, SDH, Na^+-K^+-ATPase, $Mg2^+$-ATPase and Ca^{2+}-ATPase (from low dose), lower contents of Zn, Fe (from mid-dose), lower protein expression of FSHR and LHR (from low dose) and an increase of Cu content (from mid-dose) in the ovaries compared to controls. The authors concluded that sub-chronic exposure to aluminium chloride could damage the ovarian structure, suppress energy supply in the ovary, inhibit ovulation and corpus luteum development, resulting in sterility (Fu et al. 2014). LOAEL was of 64 mg/kg bw per day of aluminium chloride, equivalent to12.96 mg Al/kg bw per day.

In a study by Martinez et al., Wistar male rats were orally exposed to aluminium chloride. The study included 2 experiments: experiment 1 with a control group and rats exposed over 60 days to 1.5 or 8.3 mg Al/kg bw per day through drinking water and, experiment 2 with a control group and rats exposed over 42 days to 100 mg Al/kg bw per day. In these experiments aluminium was detected in germinative cells and even low concentrations in testes could alter spermatogenesis and sperm quality. In fact, aluminium increased oxidative stress in the reproductive organs and caused inflammation in testis (Caroline Silveira Martinez et al. 2017b). The LOAEL was of 1.5 mg Al/kg bw per day.

Male Wistar rats were orally exposed, by gavage, to aluminium chloride at concentrations of 0, 6.7×10^{-5}, 3.35×10^{-4}, 10, and 40 mg/kg bw per day for 112 days. In this study, rats exposed to doses from 6.7×10^{-5} mg/kg bw per day had lower testis, parenchymal and epididymal weight and lower testosterone concentrations than control rats. No significant difference was reported between the low and the high exposure groups for these parameters. A significant lower sperm motility was observed in the highest dose group (40 mg/kg bw per day). Aluminium exposure did not alter the histology of testis and epididymis and sperm morphology (Mouro et al. 2018).

In another study, male Wistar rats (n=4 per group) were administered, through gavage for 90 days, 0 or 4.2 mg/kg bw per day of aluminium chloride or 1 g ethanol/kg bw per day or both. Rats exposed orally to aluminium or ethanol had a loss of normal distribution of spermatogenic cells in the seminiferous tubules and few fragmented sperms in the lumen. More adverse effects were observed in rats exposed concomitantly to ethanol and aluminium and, the author concluded that ethanol increases the impact of aluminium on testis (Ghosh, Kant Sharma, et Yadav 2021a). LOAEL was of 4.2 mg/kg bw per day of aluminium chloride, 0.85 mg Al/kg bw per day.

In another study of Gosh et al., female Wistar rats (n=4 per group) were also exposed orally and for 3 months, through gavage, to 0 or 4.2 mg/kg bw per day of aluminium chloride with or without 1 g ethanol /kg bw per day. A significant increase of atretic follicles with degenerated ova and vacuolation were seen in the ovary of aluminium treated rats in addition to the rupture of zona pellucida in oocyte. In this study, authors also observed that ethanol increased the impact of aluminium on the ovary (Ghosh et al. 2021b). LOAEL was of 4.2 mg/kg bw per day of aluminium chloride, equivalent to 0.85 mg Al/kg bw per day.

Multigeneration studies

Hirata-Koizumi et al., conducted a multigeneration GLP-compliant study to evaluate the effect of aluminium on reproduction and development. Twenty-four male and 24 female Crl:CD (SD) rats were given, through water, 0, 120, 600 or 3000 mg/L of aluminium sulphate from the age of 5 weeks for 10 weeks prior to mating, during mating and gestation (Generation F0). Males were then sacrificed, and females were also exposed through weaning and then sacrificed. At weaning, 24 male and 24 female pups were considered as generation F1 and were given the aluminium sulphate for 10 weeks prior to mating, during mating and gestation, and, for the females, through weaning, same exposure scheme as F0. Aluminium exposure from the diet and drinking water were reported. At the three aluminium sulphate concentrations, a decrease in water consumption was observed and linked to the pH of water. It was associated with a decrease of food consumption in the 600 and 3000 mg/L groups and a decrease of body weight in the 3000 mg/L group. There was a decrease in preweaning body weight gain in the F1 & F2 pups in the 3000 mg/L group in addition to a decrease in the liver and spleen weight at weaning. In addition, at 3000 mg/L there was a slight delay in the vaginal opening. On another hand, aluminium did not cause changes in other reproductive and developmental parameters and no developmental neurobehavioral toxicity was reported. The NOAEL was considered to be 600 mg/L equivalent to 41 mg/kg bw per day of aluminium sulphate. Considering the intake from food and drinking water it was 8.06 mg Al/kg bw per day (Hirata-Koizumi et al. 2011b). The LOAEL was considered to be 3000 mg/L (188 mg/kg bw per day) of aluminium sulphate equivalent to 31.2 mg Al/kg bw per day considering intake from food and water.

Another two-generation GLP-compliant study was conducted by Hirata-Koizumi et al. It included 24 male and 24 female Crl:CD(SD) rats (F0) who were exposed to either 0, 50, 500 or 5000 mg/L of aluminium ammonium sulphate through drinking water at 5 weeks of age for 10 weeks. Afterwards female rats were mated with males from the same dosage group. F0 male rats were killed after parturition and F0 females were necropsied after weaning of their offsprings but were administrated aluminium ammonium sulphate during mating, gestation and lactation periods. At weaning, 24 male and 24 female pups were identified to be generation F1 and were exposed as generation F0, to aluminium ammonium sulphate for 10 weeks before mating, during mating and gestation and during weaning for females. Aluminium exposure from diet was reported. A decrease in water consumption was reported in all treated groups but was related to the low pH of drinking water. At 5000 mg/L there was a transient body weight decrease in parental rats. Female and male reproductive performance were not affected by aluminium treatment (oestrous cycle, copulation, fertility index, precoital interval, gestation length, number of implantations, number of pups delivery, delivery index, sperm parameters), changes in reproductive organs were not reported and, in F1 and F2 pups there were no malformations, sex ratio or viability difference with the control group. A decrease in body weight was reported in male and female F1 pups on PND 21 and 14 & 21, respectively and on PND 26 in F2 pups (The reduced preweaning body weight gain might be due to decrease in water consumption).

However, there was no difference in body weight at birth between control groups and aluminium treated groups. Vaginal opening was delayed in F1 female pups at 5000 mg/L; no differences were reported regarding time of preputial separation in F1 male pups. In F1 and F2 weanlings, there was a decrease in liver, spleen and thymus weight at 5000 mg/l without histopathological changes. There was no aluminium treatment effect on locomotor activity, righting reflex and negative geotaxis reflex. The authors considered the NOAEL to be of 500 mg/L of aluminium ammonium sulphate equivalent to 33.5 mg/kg bw per day expressed as aluminium 3.81 mg/kg bw per day. Considering diet aluminium income, the calculated total aluminium level was of 5.35 mg Al/kg bw per day (Hirata-Koizumi et al. 2011a). The LOAEL was considered to be 5000 mg/L of aluminium ammonium sulphate; considering combined income from food and drinking water, it was 305 mg/kg bw per day and 36.3 mg Al/kg bw per day.

- **Gerbils**

A recent study aimed at assessing the effect of prenatal aluminium exposure on gerbils' prostate. Pregnant gerbils (*Meriones unguiculatus*) were orally exposed, by gavage, to 0 or 100 mg/kg bw per day of aluminium chloride (delivering 20.2 mg Al/kg bw per day; 1/35 LD50) during Gds 17 to 24. Following birth, males and female gerbils were separated and euthanized at either PN1 or PN90 (8 pups per group). A decrease in the body weight of PN1 males and females, a reduced anogenital distance of PN1 females, changes in the prostate developmental patterns of PN1 animals causing an increase in proliferative status and a decrease in the immunostaining of androgen receptor were reported in the aluminium exposed groups. These effects were permanent as some were also observed in the adult gerbils (L. S. Gomes et al. 2019). The LOAEL was of 20.2 mg Al/kg bw per day.

The authors conducted another study aiming to assess the effect of aluminium neonatal exposure on the male and female paraurethral gland of gerbils. Male and female gerbils (8 per group) were exposed, by gavage, to 0 or 10 mg/kg bw per day of aluminium chloride (delivering 2.02 mg Al/kg bw per day; 1/345 LD50), from day 1 to day 14 postnatal. Aluminium caused morphological changes in the ventral male paraurethral gland (intensified prostate branching morphogenesis with greater length, number and area of prostatic epithelial buds and, increased immunostaining of the androgen receptor) and in the female paraurethral gland (up regulation of the androgen receptor and oestrogen receptor α) and altered the prostate hormonal regulation of males and females (L. D. S. Gomes et al. 2020). The LOAEL was of 2.02 mg Al/kg bw per day.

Male and female gerbils (*Meriones unguiculatus*) orally exposed over 30 days to 10 mg/kg bw per day to aluminium chloride showed toxic effect on their paraurethral gland and gonads (Da Silva Lima et al. 2020). In another study, with the same exposure design, male gerbils had a paraurethral gland increased cell proliferation, glandular hyperplasia, increased secretory activity and greater androgen receptor immunoreactivity when euthanised one day after the aluminium treatment. When gerbils were euthanised 30 days after the end of the treatment, a partial recovery of the prostate were observed in males, however, in females, 30 days recovery were not enough (Da Silva Lima et al. 2022). LOAEL for both studies was of 10 mg/kg bw per day of aluminium chloride, 2.024 mg Al/kg bw per day.

- **Rabbits**

Four New Zealand rabbit groups were given through gavage, ascorbic acid (40 mg /kg bw per day) or aluminium chloride (34 mg/kg bw per day) or ascorbic acid & aluminium chloride. Results showed that rabbits exposed to 34 mg/kg bw per day of aluminium chloride over 16 weeks had a significant decrease in feed intake, body weight, relative weights of testes and epididymis, means of semen ejaculated volume, sperm concentration, total sperm output, sperm motility, total sperm ejaculate and libido (Yousef, El-Morsy, et Hassan 2005). LOAEL was of 34 mg/kg bw per day of aluminium chloride equivalent to 6.8 mg Al/kg bw per day.

- **Guinea pigs**

Hartley guinea pigs inhaling 0.065, 0.65 or 6.5 mg Al/m^3 as aluminium chlorohydrate over 6 months had no histological changes in their reproductive tissues (Steinhagen, Cavender, et Cockrell 1978). The NOAEL is of 6.5 mg Al/m^3.

- **Dogs**

Male (n=4) and female (n=4) beagle dogs were fed diets containing 0, 3 000, 10 000 or 30 000 mg basic sodium aluminium phosphate per kg over 26 weeks. This exposure is equivalent to 4, 10, 27 or 75 and 3, 10, 22 or 80 mg Al/kg bw per day for male and female dogs respectively. In the high dose group, males had a decrease in food

consumption and in body weight associated with testicular changes. NOAEL for male dogs was considered to be 27mg Al/kg bw per day (Pettersen et al. 1990).

Table VII.59: Animal studies on aluminium reproductive toxicity

Strain	Duration and exposure route	Dose	Aluminium Compound	Endpoint	NOAEL	LOAEL	Reference
Mice							
BALB/c (n≈ 6 per group)	Gd7-Gd16 Gavage	0 (ip), 200, 300 mg/kg/day	Aluminium chloride	Increased incidence of resorptions		41 mg Al/kg bw per day	Cranmer et al. (1986)
Swiss-Webster (n= 16 per group)	Gd1-Ld21 In the diet	25 (control), 500 or 1000 µg Al/g diet	Aluminium lactate	Altered gestational length from 500 µg Al/g diet		155 mg Al/kg bw per day	Donald et al. (1989)
Swiss-Webster (Control: n=14, Al group: n=9)	Gestation and lactation In diet	25 (control) or 1000 µg Al/g diet	Aluminium lactate	No effects on litter size, birth weight, crown-rump length, or sex ratio	250 mg Al/kg bw per day		Golub et al. (1992a)

Strain	Duration and exposure route	Dose	Aluminium Compound	Endpoint	NOAEL	LOAEL	Reference
Rats							
Fischer-344 (n=20 per group)	6 months (5d/wk and 6hr/d) Inhalation	0, 0.065, 0.65 or 6.5 mg Al/m^3	Aluminium chlorohydrate	Histological changes in reproductive tissues	6.5 mg Al/m^3		Steinhagen et al. (1978)
Sprague-Dawley (number not reported)	Males: 60 days prior mating Females 14 days prior to mating till end of lactation Gavage	0, 180, 360 or 720 mg/kg bw per day	Aluminium nitrate nonahydrate	No reproductive toxicity (male and female fertility)	52 mg Al/ kg bw per day		Domingo et al. (1987a)
Wistar (n=10 per group)	120 days in drinking water	0, 64.18, 128.36 or 256.72 mg/kg bw per day	Aluminium chloride	Lower androgen receptor protein expression and mRNA expression		13 mg Al/kg bw per day	Sun et al. (2011)
Wistar (n=10 per group)	120 days in drinking water	0, 64.18, 128.36, or 256.72 mg /kg bw per day	Aluminium chloride	Decrease in serum levels of oestrogen, progesterone, FSH and LH		13 mg Al/kg bw per day	Wang et al. (2012)
Wistar (n= 10 per group)	120 days in drinking water	0, 64.18, 128.36 or 256.72 mg/kg bw per day	Aluminium chloride	Increase in sperm malformation rate		13 mg Al/kg bw per day	Zhu et al. (2014)
Wistar (n= 20 per group)	120 days in drinking water	0, 64, 128 or 256 mg/kg bw per day	Aluminium chloride	Lower protein expression of FSHR and LHR and lower energy supply in the ovary		12.96 mg Al/kg bw per day	Fu et al. (2014)
Wistar (n= 6 per group)	60 days Drinking water	1.5 or 8.3 mg Al/kg bw per day	Aluminium chloride	Alteration of spermatogenesis and sperm quality		1.5 mg Al/kg bw per day	Martinez et al. (2017b)
	42 days Gavage	100 mg Al/kg bw per day					
Wistar (n= 5 per group)	112 days Gavage	0, 6.7×10^{-5}, 3.35×10^{-4}, 10, and 40 mg/kg bw per	Aluminium chloride	Lower testis, parenchyma and epididymal weight and lower testosterone concentrations		6.7×10^{-5} mg/kg bw per day	Mouro et al. (2018)
Wistar (n=4 per group)	Gavage for 90 days	0 or 4.2 mg/kg bw per day	Aluminium chloride	Loss of normal distribution of spermatogenic cells in the seminiferous tubules and few fragmented sperms in the lumen		0.85 mg Al/kg bw per day	Gosh et al. (2021)

Strain	Duration and exposure route	Dose	Aluminium Compound	Endpoint	NOAEL	LOAEL	Reference
Wistar (n=4 per group)	3 months Gavage	0 or 4.2 mg/kg bw per day	Aluminium chloride	Atretic follicles with degenerated ova and vacuolation, rupture of zona pellucida in oocyte		0.85 mg Al/kg bw per day	Gosh et al. (2021)
Gerbils							
Meriones unguiculatus (n = 20 males and 20 females per group)	30 days	0 or 10 mg/kg bw per day	Aluminium chloride	Toxic effect on paraurethral gland and gonads		2.024 mg Al/kg bw per day	Da Silva Lima et al. (2020)
Meriones unguiculatus (n= 20 for each sex per group)	30 days	0 or 10 mg/kg bw per day	Aluminium chloride	Toxic effect on paraurethral gland		2.024 mg Al/kg bw per day	Da Silva Lima et al. (2022)
Rabbits							
New Zealand (n= 6 per group)	16 weeks Gavage	34 mg/kg bw per day	Aluminium chloride	Reduced semen quality		6.8 mg Al/kg bw per day	Yousef et al. (2005)
Guinea pigs							
Hartley (n= 20 per group)	6 months (5d/wk, 6hr/d) Inhalation	0, 0.065, 0.65 or 6.5 mg Al/m^3	Aluminium chlorohydrate	Histological changes in reproductive tissues	6.5 mg Al/m^3		Steinhagen et al. (1978)
Dogs							
Beagle (n= 4 for each sex per group)	26 weeks	4, 10, 27 or 75 and 3, 10, 22 or 80 mg Al/kg bw per day for male and female	Aluminium phosphate	Decrease in food consumption and in bw associated with testicular changes	27 mg Al/kg bw per day	75 mg Al/kg bw per day	Pettersen et al. (1990)

Table VII.60: Animal studies on aluminium developmental toxicity

Stain	Duration and exposure route	Dose	Aluminium Form	Endpoint	NOAEL	LOAEL	Reference
Mice							
Swiss Webster F0 (n= 16 per group) F1 (n= 4/ litter)	Gd1-Ld21 In the diet	25, 500 or 1000 mg Al/kg diet	Aluminium lactate	No effects on birth weight, peri and post-natal pup mortality	310 mg Al/kg bw per day		Donald et al. (1989)
Swiss F0 (n= 20 per group)	Gd 6 to Gd15 Gavage	0, 23, 46 or 92 mg Al/kg bw per day	Aluminium hydroxide	No signs of embryotoxicity including morphological abnormalities	92 mg Al/ kg bw per day		Domingo et al. (1989)
Swiss (DD1) F0 (n= 10-13 per group)	Gd 6 to Gd15 Gavage	0 or 57.4 mg Al/kg bw per day	Aluminium lactate	Reduced foetal body weight, cleft palate and delayed foetal ossification		57.4 mg Al/kg bw/day	Colomina et al. (1992)
Swiss (DD1) F0 (n= 10-13 per group)	Gd 6 to Gd15 Gavage	0 or 57.4 mg Al/kg bw/day	Aluminium hydroxide		57.4 mg Al/kg bw/day		Colomina et al. (1992)
Swiss (number not reported)	Gd 6 to Gd15 Gavage	0, 103 mg Al/kg bw per day	Aluminium hydroxide	No developmental effects	103 mg Al/kg bw per day		Colomina et al. (1994)
CD-1 F0 (n= 10-14 per group)	One day between Gd8-Gd12 Gavage	0 or 71 mg Al/kg bw	Aluminium nitrate	Reduced foetal body weight, reduced ossification		71mg Al/kg bw	Albina et al. (2000)
Rats							
Wistar	Gd 8 until parturition In the diet	0, 160 or 200 mg Al/kg bw per day	Aluminium chloride	Reduced pup weight on PND1		160 mg Al/kg bw per day	Bernuzzi et al. (1986)
Sprague Dawley	Gd14 to Gd21 Gavage	0, 13, 26 or 52 mg Al/kg bw per day)	Aluminium nitrate	Reduced number of litters and live pups per litter		13 mg Al/kg bw per day	Domingo et al. (1987c)
Sprague Dawley F0 (n= 10 per group) F1 (n= more than half of the foetuses)	Gd 6 to Gd 14 Gavage	0, 13, 26 or 52 mg Al/kg bw per day	Aluminium nitrate	Higher number of runt foetuses, reduced bw, delayed ossification, increase of congenital malformations and minor anomalies		13 mg Al/kg bw per day	Paternain et al. (1988)

Stain	Duration and exposure route	Dose	Aluminium Form	Endpoint	NOAEL	LOAEL	Reference
Wistar F0 (n= 6 to 12 per group)	Gd 1 to parturition In the diet	0, 20, 60 and 80mg Al/kg bw per day	Aluminium chloride	Reduction of pup weight, higher postnatal mortalities	20 mg Al/kg bw per day	60 mg Al/kg bw per day	Bernuzzi et al. (1989a)
Wistar F0 (n= 6 to 10 per group)	Gd 1 to parturition In the diet	0, 9, 18 and 36 mg Al/kg bw per day	Aluminium lactate	Reduction of pup weight, higher postnatal mortalities	18 mg Al/kg bw per day	36 mg Al/kg bw per day	Bernuzzi et al. (1989a)
Wistar rats F0 (n= 6 to 9 per group)	Gd1-Gd7 or Gd1-Gd14 or Gd1-partution In the diet	0 or 400 mg Al/kg bw per day	Aluminium lactate	No effect of on the litter size, mortality rate or weight of pups	400 mg Al/kg bw per day		Muller et al. (1990)
Wistar F0 (n= 18-19 per group)	Gd 6 to 15 Gavage	0, 66, 132 or 264 mg Al/kg bw per day	Aluminium chloride	No embryotoxicity		264 mg Al/kg bw per day	Gomez et al. (1990)
Sprague Dawley F0 (n= 11 to 17 per group) F1 (n= 8 per litter)	15-day premating to post-weaning In water	0, 50 or 100 mg Al/kg bw per day + citric acid	Aluminium nitrate nanohydrate	Delay in vaginal opening	50 mg Al/kg bw per day		Colomina et al. (2005)
Crl:CD (SD)	Multigenerational GLP-compliant study In water	0, 120, 600 or 3000 mg/l	Aluminium sulphate	Decrease in preweaning bw, decrease in liver & spleen weight at weaning, delayed vaginal opening	8.06 mg Al/kg bw per day	31.2 mg Al/kg bw per day	Hirata-Koizumi et al. (2011b)
Crl:CD (SD)	Multigenerational GLP-compliant study In water	0, 50, 500 or 5000 mg/l	Aluminium sulphate	Delayed vaginal opening in F1 female, decrease in liver, spleen and thymus weight	3.81 mg/kg bw per day	36.3 mg Al/kg bw per day	Hirata-Koizumi et al. (2011a)
Gerbils							
Meriones unguiculatus F0 (n=10 per group) F1 (n=8 per group)	Gd 17 to 24 Gavage	0 or 20.2 mg Al/kg bw per day	Aluminium chloride	Decrease in bw of PN1, changes in the prostate developmental patterns of PN1		20.2 mg Al/kg bw per day	Gomes et al. (2019)
Meriones unguiculatus (n= 8 of each sex per group)	PND 1 to 14 Gavage	0 or 2.02 mg Al/kg bw per day	Aluminium chloride	Morphological changes in the ventral male prostate and female prostate		2.02 mg Al/kg bw per day	Gomes et al. (2020)

Table VII.61: Animal studies on aluminium neurodevelopmental toxicity

Strain	Duration and exposure route	Dose	Aluminium Form	Endpoint	NOAEL	LOAEL	Reference
Mice							
Swiss Webster (F0= 16 per group) F1 (n= 4/ litter)	From conception to Ld21 In the diet	25 (control), 500 or 1000 µg Al/g diet	Aluminium lactate	Decreased forelimb, increased hindlimb grip strength, increased foot splay in weanlings		155 mg Al/kg bw per day	Donald et al. (1989)
Swiss-Webster F0 (n= 9-14 per group) F1 (n= 2 pups per litter)	G: Gd1 -Gd19 or G+ D: Gd1 - Ld21 or L: Ld1-Ld21 In the diet	25 (control) or 1000 µg Al/g diet	Aluminium lactate	Decrease forelimb grip strength (G), increase in hindlimb grip strength and tail withdrawal times (gestation and lactation groups), increase in negative geotaxis latency (L)		250 mg/kg bw per day	Golub et al. (1992a)
Swiss-Webster (n= 8 males and females per group)	From conception to weaning In the diet	7 (control), 500 or 1000 µg Al/g diet	Aluminium lactate	Decrease in forelimb and hindlimb grip strength and in the startle response		155 mg Al/kg bw per day	Golub et al. (1995)
Swiss Webster (n= 8 per group)	From conception to PND 35 In the diet	7 (control), 500, or 1000 µg Al/g diet	Aluminium lactate	Performance of the Delayed spatial Alternation task	330 mg Al/kg bw per day		Golub and Germann (1998)
Swiss Webster (n= 18 per group)	Conception – month 24 of age In the diet	7 (control) or 1000 µg Al/g diet	Aluminium lactate	Decreased forelimb and hindlimb strength, decreased thermal activity		100 mg Al/kg bw per day	Golub et al. (2000)
Swiss Webster (n = 20 per group)	From conception to PND 35 In the diet	7 (control), 100, 500, or 1000 µg Al/g diet	Aluminium lactate		26 mg Al/kg bw per day	130 mg Al/kg bw per day	Golub and German (2001)
Swiss-Webster (n= 21 pups per group)	From Gd1 to PND15 In water	0, 300 or 600 mg Al/kg bw per day	Aluminium chloride	Deficit in locomotor activity, learning capacity and cognitive behaviours		300 mg Al/kg bw per day	Abu Taweel et al. (2012)

Strain	Duration and exposure route	Dose	Aluminium Form	Endpoint	NOAEL	LOAEL	Reference
Rats							
Sprague Dawley F0 (n= 11-17 per group) F1 (n= 1 male & 1 female of each litter)	15 days premating and from Gd1 to Ld21 In water	0, 50 or 100 mg Al/kg bw per day (+citric acid added)	Aluminium nitrate	Decreased forelimb grip	50 mg Al/kg bw per day	100 mg Al/kg bw per day	Colomina et al. (2005)
Sprague Dawley (n=17-21 per group)	Conception- 1 or 2 years old In water	0, 50 or 100 mg Al/kg bw per day (+citric acid added)	Aluminium lactate	Performance of water maze test	100 mg Al/kg bw per day		Roig et al. (2006)
Wistar (n= 10 per group)	From conception to 4 months age In water	0 or 3 g/l $AlCl_3$	Aluminium chloride	Increased GFA-P astrocytes in brain, increased anxiety, reduced locomotor activity		600 mg Al/l	Erazi et al. (2010)
Sprague Dawley F0 (n= 20 per group) F1 (n=4 of each sex per litter)	From Gd6 to PND 364 In water	0, 30, 100 or 300 mg Al/kg bw per day	Aluminium citrate	Decrease in hindlimb and forelimb grip strength (+other effects)	30 mg Al/kg bw per day	100 mg Al/kg bw per day	Poirrier et al. (2011)
Wistar (n= 8 per group)	From parturition to 3-month age In water	0, 0.2, 0.4 or 0.6% of $AlCl_3$	Aluminium chloride	Pathological changes in neuronal and synaptic ultrastructure and impaired spatial memory ability		400 mg Al/l	Zhang et al. (2013)

4.9 Macrophagic myofaciitis

Macrophagic myofasciitis (MMF) is a rare inflammatory condition characterized by specific muscle lesions infiltrated by macrophages containing aluminium crystal inclusions and was reported for the first time in 1982 (Mrak 1982). This lesion generally results from aluminium adjuvant depots after vaccine injection. Though the duration of this phenomenon after vaccination is not known and could be variable from an individual to another, there is no indication that it is pathologic.

More recently French authors published from 1998 to 2023, clinical and experimental studies with the aim to establish an association between MMF and a systemic syndrome diversely associating diffuse myalgias, arthralgias, fatigue, muscle weakness, fever and cognitive alterations' (Gherardi et al. 1998; Eickhoff et Myers 2002; HCSP 2013; Goullé et Grangeot-Keros 2020). However, the published studies suffer several methodologic flaws: inclusion criteria of the patients in the cohort are not presented; the relative frequencies of the signs and symptoms

constituting the syndrome, as well the sex ratio of the patients in the cohort are broadly fluctuating from a publication to another. The cohort is constituted of patients with a biopsy of the deltoid showing a MMF and also complaining for the systemic syndrome described above. There is (as ethically expected) no control group with no systemic complaint and results of a deltoid biopsy.

Finally, MMF as a local reaction after intramuscular injection of an aluminium-adjuvanted vaccine is a largely documented phenomenon. There is no demonstration that it is or can be causally associated with a systemic syndrome or illness. Strong arguments against a causal link are that this association has been quasi-exclusively described in France and by the same authors and rarely in children, though the latter constitute the fraction of the population with the bigger exposure to aluminium adjuvanted vaccines.

Evaluations by Afssaps (2004) Scientific Committee and by HCSP (2013) both concluded that there is no evidence that MMF following intramuscular injection of aluminium-adjuvanted vaccines is causally associated with one or more systemic manifestations (Afssaps 2004; HCSP 2013).

In 2022, the US Centers for Disease Control and Prevention (CDC) acknowledged an observational study in which a possible association between exposure to vaccine aluminium and the subsequent development of persistent asthma in a cohort of children was identified (Daley et al. 2023). This finding is considered by the CDC to be a health signal but does not call into question the assessment of risk secondary to aluminium exposure following the vaccination schedule (Mitkus et al. 2011).

4.10 Genotoxicity

4.10.1 *In vitro* studies

In studies documented in EFSA's report, Aluminium ion (Al^{3+}) was proved to interact with DNA *in vitro* by binding to phosphate oxygen. Several aluminium compounds showed negative results in bacterial mutagenicity assays and in mammalian cells in vitro and, others produced DNA damage (EFSA 2008). These studies are reported below.

Aluminium lactate at concentration of 1.8 - 5.5 micromol/plate gave negative response in the reverse mutation test using various *Salmonella typhimurium* strains (Gava et al. 1989). Negative results were also observed for aluminium fluoride at 0.02-119 micromol/plate (Shimizu et al. 1985), aluminium silicate at 0.96 - 38.5 micromol/plate (Zeiger et al. 1987), sodium aluminium silicate at 0.36 - 108.1 micromol/plate (Prival, Simmon, and Mortelmans 1991), aluminium chloride hexahydrate at 0-100 nanomol/plate (Marzin and Phi 1985), aluminium sulphate up to 5000 µg/plate (ECHA, Registration dossier) and aluminium chloride at concentrations of 0.3 and 3.0 mg/l in an assay carried out in suspension culture (Ahn and Jeffery 1994).

In other studies, bacterial tryptophan reverse mutation assay using *Escherichia coli WP2* strain, showed negative responses of aluminium chloride, aluminium fluoride, calcium aluminosilicate and sodium aluminium silicate to induce gene mutations (Seo, and Lee 1993; Shimizu et al. 1985; Prival, Simmon, and Mortelmans 1991).

No mutagenic activity was induced by aluminium oxide, aluminium chloride or aluminium sulphate at concentrations of 1-10 mM in the rec-assay using *Bacillus subtilis* strains (ATSDR 2006).

In L5178Y mouse lymphoma assay, no forward mutations were detected with aluminium chloride at concentrations up to 625 µg aluminium chloride/ml (Oberly, Piper, and McDonald 1982).

The induction of micronuclei in human lymphocytes was increased following exposure to aluminium sulphate (exposure for 48h after PHA stimulation) (Migliore et al. 1999) and to aluminium chloride (with a decrease at high dose correlated to an increase of apoptosis) (Banasik et al. 2005). Paz et al. have also observed a significant increase in the quantity of micronucleus in human lymphocytes (from the peripheral blood) exposed to aluminium chloride at concentrations of 5 µM, 10 µM and 20 µM (Paz et al. 2017).

Effects of aluminium chloride (1, 2, 5, 10 and 25 µg/ml) on DNA damage and apoptosis in human lymphocytes were assessed using a comet assay. The results showed a dose-dependent increase in DNA damage up to the dose of 10 µg/ml of aluminium chloride and declined at 25 µg/ml due to increase in apoptosis (Lankoff et al. 2006). The authors then evaluated the effect of aluminium on DNA repair and found that aluminium chloride treated cells had a decreased capacity of DNA repair compared to controls (Lankoff et al. 2006). Lima et al. have also observed

aluminium induced DNA damage in human lymphocytes in addition to structural chromosomal aberrations (Lima et al. 2007).

In the study by Tenan et al. (2021), in V79 hamster lung fibroblasts exposed to aluminium, dose-dependent increases in DNA double strand breaks, and chromosome numerical abnormalities (aneuploidy) as well as arrest in the G2/M phase of the cell cycle, were observed. Additionally, during mitosis, abnormal multipolar mitotic spindles were detected (SCCS 2023).

4.10.2 *In vivo* studies

Since the aluminium salts are able to induce an oxidative stress, they could possibly induce mutagenicity *in vivo* by this mechanism of action.

In vivo genotoxicity studies in rodents showed a clastogenic potential of aluminium, indicating that it can cause chromosomal damage (EFSA 2008).

In a study by Manna and Das (1972), mice intraperitoneally injected with aluminium chloride exhibited a significant increase in chromosome aberrations in the bone marrow. However, no clear dose-response relationship was observed (ATSDR 2008; EFSA 2008).

As stated in EFSA (2008), the administration of aluminium sulphate (17, 22, 28, 43, 85 or 172 mg Al3+/kg bw) or aluminium potassium sulphate (28 or 43 mg Al^{3+}/kg bw) to rats by gavage, daily over 21 days, led to a dose-dependent inhibition of cell division and an increase in chromosome aberrations, in the bone marrow (Roy, Sharma, and Talukder 1991). Furthermore, a dose-dependent induction of micronuclei was observed in the bone marrow of mice injected intraperitoneally with aluminium sulphate (2 doses, 24 hours apart). Aluminium sulphate also induced sister chromatid exchanges in the bone marrow of mice injected intraperitoneally, in a dose related manner (Roy, Dhir, and Sharma 1992).

In a more recent study by Paz et al., Swiss mice (n= 8 per group) were orally administered hydrated aluminium chloride at 0, 49, 98 or 161 mg Al/kg bw to assess aluminium's possible genotoxic activity using micronucleus test. The study included a negative control and a positive control group. An increase of micronucleus number was observed in all aluminium concertation groups and significant alterations in all the evaluated organs were identified and verified by the presence of irreversible lesions (Paz et al. 2017).

In another study, Sprague Dawley rats (n= 8 per group) were gavaged with 0 or 2000 mg/L aluminium (as aluminium chloride) 5 days/week for 90 days with or without N-nitroso-N-methylurea (NMU) induction of breast cancer. A higher number of micronucleus count in peripheral blood erythrocytes was observed following the exposure to +2000Al/−NMU and −Al/+NMU treatments indicating that treatment containing only aluminium can independently cause genotoxicity in rats. Furthermore, comets were observed after 10 and 15 days in the Comet Assay in rats receiving the +2000AL/-NMU treatment (García-Alegría et al. 2020).

As stated in the recent opinion by SCCS, in the study by Mandriota et al. (2020), normal mouse mammary epithelial cells after long-term culture in the presence of aluminium chloride formed tumours and metastases when injected into syngeneic and immunocompetent BALB/cByJ mice. As was shown by the authors aluminium chloride rapidly increased chromosomal structural abnormalities in the cultured cells (SCCS 2023).

In the study of Jalili et al., acute exposure to aluminium chloride (25 mg/kg bw through gavage) induced slight but non-significant oxidative DNA damage in peripheral blood lymphocytes of Sprague-Dawley rats (n= 5 per group). No increase of micronuclei in both bone marrow cells and in colon was observed (Jalili et al. 2020).

EFSA (2008) stated that aluminium had genotoxic effects at high level of exposure, not relevant for human exposure through the diet. Nevertheless an *in vivo* mammalian erythrocyte micronucleus test combined with in vivo mammalian alkaline comet assay with aluminium oxide has been requested by ECHA, but the test has not been performed yet. The results of this study may better clarify the assessment on the potential of aluminium salts to induce genotoxicity, as stated by SCCS in its latest opinion (SCCS 2023).

4.11 Carcinogenicity

4.11.1 Human data

Based on the quantification of aluminium in breast cancer tissues, a potential link between antiperspirants and breast cancer was hypothesized, supported by the observation of a high incidence of breast cancer in the upper outer quadrant, adjacent to the area of typical application of deodorants and/or antiperspirants (Darbre 2005; Exley et al. 2007). Epidemiological studies had assessed the association between exposure to aluminium through cosmetics containing aluminium and the risk of breast cancer but none of them established a causal link between aluminium exposure and breast cancer. It has been suggested that a reverse causal effect cannot be excluded, implying that the breast tumour could accumulate aluminium (Linhart et al. 2017).

The Scientific Committee on Consumer Safety (SCCS) has conducted safety assessments of aluminium exposure through cosmetic products on four occasions since 2014 (in 2014, 2020, 2023, and 2024). It concluded that despite the known genotoxic effects of aluminium, which may potentially contribute to the development of breast cancer, the existing data from both animal and epidemiological studies are presently insufficient to definitively establish a causal relationship between aluminium exposure and the risk of developing breast cancer.

4.11.2 Animal data

No reliable studies regarding cancer effects were identified in animals following acute or intermediate duration inhalation of aluminium or its compounds.

Oneda et al., did not report an increase of tumours incidence or other proliferative lesions in B6C3F1 mice ingesting aluminium potassium sulphate through diet at doses of 1.0, 2.5, 5.0 and 10.0% (w/w) over 20 months (the study included a control group). In this study, hepatocellular carcinoma's incidence was significantly decreased in the group of high-dose males (Oneda et al. 1994).

Following oral exposure of Swiss CD mice to aluminium potassium sulphate in drinking water (5 ppm Al, equivalent to 1.2 mg Al/kg bw/day) from weaning through their lifetime (mean lifespans were of 533 days), a significant increase in the incidence of gross tumours was observed in females (46.3% in the aluminium exposed group vs 29.8% in the control group). In addition, the incidence of lymphoma leukaemia was also significantly increased in females (10/41 vs 3/47) (Schroeder et Mitchener 1975b). No significant increase of tumour incidence was observed in male mice.

In another study, Long-Evans rats were exposed to aluminium potassium sulphate in their drinking water (5 ppm Al, equivalent to 0.6 mg Al/kg bw/day) from weaning through their lifetime (2 years). Only males presented an increase in the incidence of gross tumours (52% in the aluminium exposed group vs 15% in the control group). Of the tumours, six were malignant in the aluminium exposed group vs 2 in the control group (Schroeder et Mitchener 1975a). No significant increase of tumour incidence was observed in female rats.

In both studies, only one dose of aluminium was used and, the type of tumours and the organs where they were found were not mentioned. Thus, it could not be determined if this increase incidence was dose dependent. The levels of aluminium in the base diet were mentioned but the diet was low in trace elements.

In a study by Pigott et al., male and female rats (Wistar) did not show an increase in cancer rate following whole-body inhalation of 2.18-2.45 mg Al/m^3 as alumina fibres (≈ 96% aluminium oxide, Saffil fibres & aged Saffil fibres with a median diameter ranging between 3.0-3.3 µm) over 86 weeks (5 d/wk and 6hr/d) (Pigott, Gaskell, et Ishmael 1981).

Four groups of female Sprague Dawley rats (n= 8 per group) were exposed to 0 or 2000 mg/L aluminium (as aluminium chloride) by gavage 5 days/week for 90 days with or without N-nitroso-N-methylurea (NMU) induction of breast cancer: −Al/−NMU; +2000Al/+NMU; +2000Al/−NMU; −Al/+NMU. The dose of aluminium is equivalent to 10 mg Al/kg bw per day. The group +2000Al/−NMU had a significantly higher aluminium concentration in the mammary gland. In the aluminium-treated groups there was a moderate intraductal cell proliferation (hyperplasia) but no cancer development; cell proliferation was minimal in the –Al/+NMU group (García-Alegría et al. 2020).

4.12 Sensitive population

People suffering from kidney failure are the main population at risk of aluminium overload and are therefore, more sensitive to aluminium toxicity (Krewski et al. 2007; ATSDR 2008).

5 Biomarkers of exposure and biometrology

The scientific literature search failed to identify any biomarkers of early effects relevant for the monitoring the aluminium exposure in the general population and occupational settings. Therefore, these biomarkers will not be developed further.

5.1 Measurement of aluminium in biological samples

Aluminium can theoretically be measured in all biological liquids, tissues, or excreta (e.g.: blood, serum, urine, cerebrospinal fluid, semen, milk, saliva, bone, hair or even nails) (ATSDR 2008).

Analytical methods for aluminium determination

Samples can be analysed after dilution (water, Triton®, EDTA, nitric acid) or after microwave-assisted acidic mineralisation. Nitric acidification is sufficient, for most of the metals present in urine, to ensure good preservation for a few weeks at +4°C.

After sample homogenization, aliquots are dispensed into clean tubes and either refrigerated (for preservation of less than two weeks) or frozen (at - 20°C, when the preservation time could be of more than 2 weeks) for subsequent analysis. If the sample is frozen, defrosting is followed by homogenization and centrifugation. The sample must be properly mixed before dosing as, for example, aluminium phosphate tends to precipitate in urine (WHO 1996; Labat 2010; San Martín, Bauçà, et Martinez-Morillo 2022).

Aluminium determination remains a challenge, regardless of the techniques used or matrices studied, because of the ubiquity of this element and the contamination risks associated with it. In fact, the majority of analytical errors are due to contamination of the sample by aluminium from ambient air, laboratory equipment or reagents used for sampling and sample preparation. It is essential to use high-purity standards and reagents, and type 1 ultrapure water.

Blank determination from pre-washed sample containers is recommended to ensure that the cleaning protocols and pre-analytical steps avoided aluminium contamination. The laboratory's environment should be kept as clean as possible.

Numerous analytical methods exist for aluminium concentration measurements in biological materials. Nevertheless, due to their insufficient detection limits or significant number of interferences, some of these techniques are no longer used. These include UV-visible absorption spectroscopy, fluorescence spectroscopy, flame atomic absorption spectrometry, X-ray fluorescence spectrometry, and neutron activation analysis. At present, inductively coupled plasma mass spectrometry (ICP-MS) is the method of choice for measurement of aluminium, as it has shown to offer the best limits of quantification, selectivity and robustness (San Martín, Bauçà, et Martinez-Morillo 2022).

The main analytical methods for the measurement of aluminium are described below. It should be noted that the aim of this section is not to recommend an analytical method for the measurement of aluminium, but to inform on specific metrological parameters and to present the advantages and limitations of each method.

- **Electrothermal Atomic Absorption Spectrometry (ETAAS) or Graphite furnace atomic absorption spectrometry (GFAAS)**

In this technique, the sample introduced into the graphite furnace undergoes the successive steps of matrix dehydration and mineralization, element atomization, and pyrolysis. The last step serves to clean the furnace to prevent inter-sample contamination.

To improve selectivity and eliminate interference, this technique requires the use of background correctors, Zeeman correction being the preferred method, preventing molecular interference and conferring high specificity. Alternatively, matrix modifiers are used to displace chlorine atoms and prevent the formation of volatile aluminium chloride in the pre-atomization stages. However, matrix modifiers can be a source of contamination, so their use is not advisable.

One of the main sources of contamination with this technique is atmospheric dust, particularly in the sampler cups, a type of contamination that appears to be almost non-existent with ICP.

Advantages of this technique include its simplicity, sensitivity and low cost. In fact, it does not require sample pre-treatment, and only a small sample volume is required. Detection limits in urine and blood samples are around 1-2 µg/L (0.04-0.07 µmol/L) (ANSES 2003; ATSDR 2008; San Martín, Bauçà, et Martinez-Morillo 2022). The LOQ was reported to be of 2 µg/L (INRS Biotox).

- **Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES):**

This technique is based on the production of excited atoms within an argon plasma (partially ionized gas with a temperature ranging from 3000 to 8000 K). The plasma is generated by an electromagnetic field produced by an induction coil connected to a high-frequency generator. Element identification and quantification are achieved by measuring the intensity of the light emitted by the elements excited in the neutral or ionized state.

While less sensitive than ETAAS, ICP-AES offers several advantages:

- Analysis speed (resulting in a significantly reduced need for re-analysis).
- Dynamic range (a larger linear range, requiring fewer dilutions).
- Specificity (less sensitive to matrix effects, allowing analysis of diverse samples without modifications of the analytical parameters).
- Multi-element capability (ANSES 2003).

The limits of detection of aluminium using ICP-AES are about 1µg/L for urine and 4µg/L for blood samples (Allain et Mauras 1979 in ATSDR 2008). The lowest LOQ was reported to be of 1µg/L (INRS Biotox).

Hence, ICP-AES is used when high sensitivity is not required, for the analysis of major components, or when the matrix is highly charged (soils, ores, alloys, etc.). It is easy to use and is faster.

Possible interferences for aluminium by ICP-AES are with Ti, Mo, Zr, Fe, U, Ce (Meggers et Corliss 1961; Burden et al. 1995; ATSDR 2008).

The most commonly used spectral lines, because they are more sensitive, are 167.020 nm (Fe interference) and 396.152 nm (Zr, Ce interferences). Interferences in urine do not seem to be problematic.

- **Inductively Coupled Plasma Mass Spectrometry (ICP-MS) / High Resolution ICP-MS:**

This technology combines two principles: ICP, a highly efficient source of ion production, and mass spectrometry for ion separation. It offers several advantages:

- Very high sensitivity.
- Enhanced detection limits: 0.1–1 µg/L (0.004–0.04 µmol/L) for most of the elements of the periodic table
- The possibility of multi-element and isotopic analysis of trace elements.

Interferences (spectroscopic and non-spectroscopic) must also be controlled (ANSES 2003; ATSDR 2008; San Martín, Bauçà, et Martinez-Morillo 2022). LOQs take into account potential interferences and background noise and the management of interference does not seem problematic.

Generally; LOQ ranged between 0.2 and 10 µg/L (INRS Biotox). In a biomonitoring study, the LOQ of aluminium in urine samples using ICP-MS was of 0.156 µg/L and the LOD 0.052 µg/L (Bertram et al. 2023).

The advantages and limitations of the analytical methods for aluminium measurement are summarised in Table VII.62 (ANSES 2003; ATSDR 2008; Wilschefski and Baxter 2019; San Martín, Bauçà, and Martinez-Morillo 2022, INRS Biotox).

Table VII.62: Overview of the advantages, limitations and LODs of the main analytical techniques used for aluminium measurement

Analytical method	Advantages	Limitations	Limit of detection	Limit of quantification
ETAAS / GFAAS	• Simplicity of sample preparation • Low sample volume • Few interferences • Sensitivity	• Single element technique • Limited analytical range	1-2 µg/L (0.04-0.07 µmol/L)	2 µg/L
ICP-AES	• Simplicity of sample preparation • Low sample volume • Mutli-element analysis • High specificity • Large analytical range	• Possible interferences • Low sensitivity	High detection limit: 1-4 µg/L	1 µg/L
ICP-MS	• Simplicity of sample preparation • Low sample volume • Multi-element analysis • Very high sensitivity • Large analytical range	• Need for high skilled expertise •	0.1–1 µg/L (0.004–0.04 µmol/L)	0.2-10 µg/L

Whatever the analytical technique used, measurement uncertainties are estimated by analytical repeatability and intermediate precision.

Laboratories must indicate the limit of detection and quantification and the uncertainties of the method used when reporting the results of aluminium analysis in the corresponding matrix.

The use of reference material, if available, is recommended for any type of biological matrix. For this end, the procedures recommended within the framework of the European projects DEMOCOPHES, HBM4EU and PARC, concerning the harmonization of biomonitoring of European populations, can be used (Schindler et al. 2014; Vorkamp et al. 2021; Zare Jeddi et al. 2022). In fact, reference materials should be incorporated into the analysis process, i.e. samples of the same matrix as the specimen with a well-known amount of biomarker concentration (close to the ones of the specimens). This guarantees reliable results. Control samples are generally commercially available certified samples.

External quality assurance schemes provide a way for laboratories to assess their performance by comparing it to that of other participating laboratories through benchmarking. Different relevant programmes including aluminium are available: the OELM External Quality Program (Occupational and Environmental Laboratory Medicine), included matrices are serum, whole blood, and urine; the UKNEQAS for Trace Elements (United Kingdom) involving serum, blood, urine, and dialysis water and fluids; the G- EQUAS (German External Quality Assessment Scheme) and certification for occupational-medical and environmental-medical toxicological analyses in biological materials, including urinary aluminium and, the QMEQAS of the Centre de Toxicologie du Québec also using several matrices like serum, blood, urine, and hair (ANSES 2003; SOCIETE FRANCAISE DE MEDECINE DU TRAVAIL 2016; San Martín, Bauçà, et Martinez-Morillo 2022; Biotox INRS[36]).

The laboratory participation in external quality control programs, and the systematic implementation of internal quality controls are determining points for obtaining COFRAC-ISO 15189 accreditation, which is a guarantee, for users, of laboratory reliability (Nübler et al. 2021).

[36] Biotox INRS: https://www.inrs.fr/publications/bdd/biotox/dosage.html?refINRS=Dosage_1 (accessed on March 2024)

5.2 Identification of biomarkers of exposure

Although aluminium can theoretically be measured in different biological liquids, tissues, or excreta, in practice, blood and urine appear as the most commonly used matrices for the routine biomonitoring of aluminium exposure, both in an occupational or an environmental context.

Though deferoxamine challenge test (measurement of aluminium excretion after deferoxamine administration) and bone aluminium concentration are probably good indicators of aluminium body burden they do not appear adapted for routine surveillance. Indeed, the challenge test is invasive and deferoxamine is not always well tolerated (R. A. Yokel 1994). Non-invasive *in vivo* bone aluminium measurement is still in the field of research (Aslam et al. 2009).

Already demonstrated to be of valuable use in forensic for several classes of substances in particular inorganic compounds, hair has multiple advantages for human biomonitoring: it is stable, easy to access for sampling, well tolerated, and reflects long-term exposure (Peña-Fernández A, González-Muñoz Mj, et Lobo-Bedmar Mc 2014). Similarly, metal concentration in toenails is a non-invasive biomonitoring tool and early warning indicator that is favoured over fingernails because of the lower risk of external contamination (Di Ciaula et al. 2020). Indeed, the measurement of aluminium in hair and nails has gained considerable interest in recent years. The use of ICP-MS provides high sensitivity and reproducibility in these matrices.

However, specifically in the context of environmental exposure, the relevance of aluminium in hair or nails as an effective biomarker of exposure remain to be established, particularly with regard to the prediction of health effects. Indeed, while measurement in these matrices offers the advantage of being non-invasive, it remains challenging to differentiate between aluminium incorporated in the hair (internal exposure) and aluminium deposited on the hair (external exposure), even with advances in technology and especially in the case of aluminium, where the substance itself is measured in the hair and nails and not a metabolite.

Most of the toxicological studies and data available concern aluminium measured in urine and/ or blood. These two matrices will be developed further in the following chapters.

5.2.1 Aluminium in urine

Many studies in workers or volunteers have demonstrated that urine aluminium levels measured at the end of a shift and after several shifts is a good biological indicator of the mean exposure of the preceding week (Lauwerys et Hoët 2001; Rossbach et al. 2006; Bertram et al. 2015; Vesa Riihimäki et Aitio 2012).

In addition, urine aluminium is possibly a good indicator of aluminium body burden, especially when measured at least 2-3 days after discontinuation of exposure (beginning of the shift) (most of the elimination of the amounts recently absorbed occurring within 2 days) (Biotox INRS[14]). There is no human data validating this hypothesis.

Actually, cross sectional and longitudinal epidemiological studies have shown an increase of the risk of neurotoxic cognitive effects, associated with the elevation of urine aluminium concentration in occupationally exposed workers (see section 4.8.1.1).

5.2.2 Aluminium in blood

Concentrations of aluminium in whole blood, erythrocytes, serum and plasma are usually considered approximately equal, and all four blood matrices could theoretically be considered for internal exposure assessment (Poddalgoda et al. 2021). However:

- Studies on the distribution of aluminium between serum (or plasma) and erythrocytes gave conflicting results (see section 4.1.2), and studies on the association of whole-blood or erythrocytes aluminium levels with health effects or aluminium external exposure are scarce. Consequently, these two matrices cannot be presently considered for aluminium exposure biomonitoring.
- Serum and plasma levels are theoretically equivalent, but anticoagulants, such as heparin or citrate, may contain aluminium. For this reason, serum should be preferred over plasma.

When measured at the end of shift with stable chronic exposure (e.g., welding), serum aluminium levels are well correlated with atmospheric exposure (Biotox INRS assessed in April 2024). A good correlation (r=0.824) was reported between circulating (serum) and excreted (urine) aluminium concentrations (V. Riihimäki et al. 2000). However, urinary aluminium is considered as a more sensitive indicator of variations of exposure and/or body burden. Indeed, for low-level occupational exposures (lower than 5 mg/m^3 of elemental aluminium), serum aluminium levels are not significantly different from those of the general population, when small variations of exposure are detectable through urinary aluminium monitoring. In addition, after the end of exposure, serum aluminium levels return to normal more rapidly than urinary aluminium concentrations (Vesa Riihimäki et Aitio 2012; INRS 2021)

In conclusion, determination of serum aluminium lacks sensitivity for revealing small variations of external exposure and/or for the biomonitoring of the body burden, especially in low exposure situations. However, serum aluminium is still the best biomarker for aluminium body burden in people with renal failure, as their aluminium internal dose can be high, and urine aluminium level is not a validated exposure indicator for those people.

Table VII.63 summarises the advantages and limitations of the most commonly used matrices for the measurement of total aluminium. Other potential matrices are not included in the table as they are rarely used and because of the lack of available literature.

Table VII.63: Overview of the advantages and limitations of the main matrices used for total aluminium measurement

Analyte	Matrix	Advantages	Limitations
Aluminium	Urine	• Non-invasive • Reflects both aluminium body burden and recent exposure • More sensitive than blood aluminium to changes in external exposure • Association established with risk of neurological effects for urine aluminium levels at the end of a shift after several shifts (see chapter 4.8.1.1)	• Influenced by impaired renal function • Intra- and inter-individual variations in urine concentration (related to water intake and excretion); those variations can be managed through adjustment on urine creatinine level, urine density or urine osmolality • High risk of external contamination of samples, at sampling time and during sample preparation and analysis
Aluminium	Whole blood, Plasma, Serum	• Reflects more recent exposure • More reliable when renal function is not normal	• Invasive • Less sensitive than urine aluminium to changes in external exposure • High risk of external contamination of samples, at sampling time and during sample preparation and analysis
Aluminium	Hair	• Non-invasive, Stable	• Challenging to differentiate internal and external exposure • Limited data on the association with health effects
Aluminium	Nails	• Non-invasive, easy to access for sampling, well tolerated	• Challenging to differentiate internal and external exposure • Limited data on the association with health effects

5.3 Factors that may influence the interpretation of aluminium measurement

Certain practices can influence the interpretation of aluminium measurements in workers or the general population by increasing aluminium levels and making the exposure not readily interpretable. Table VII.64 summarises these different factors.

Table VII.64: Factors that may influence the interpretation of aluminium measurement

Total aluminium in urine or plasma	
Medical treatment	Consumption of certain drugs containing aluminium compounds as an active ingredient or as an adjuvant could increase aluminium levels and should be avoided prior to sampling (e.g.: some antacids, buffered aspirins, antidiarrheals, etc...).
Food intake	Food contact with aluminium packaging, kitchen utensils and, aluminium films under acidic conditions can allow aluminium emission and food contamination (Krewski et al. 2007). Furthermore, fruit juices (containing citric acid which increases aluminium absorption, see section 4.1.1.2) can increase urinary aluminium levels and should be avoided in the 2 days prior to sampling (Biotox)[37].
Smoking	Even though high concentration of aluminium in tobacco are reported, ranging from 0.6 to 3.7 mg Al/g product (Exley et al. 2006), smoking did not influence aluminium concentrations in plasma and urine of occupationally non-exposed subjects (Chiba et Masironi 1992; Nisse et al. 2017). Thus, it might not impact the biomonitoring result's interpretation.
Physiological or pathological factors	As aluminium is mainly eliminated in the urine (see section 4.1.4), patients with reduced renal function, might have higher levels of aluminium in blood due to the lack of normal clearance (implying reduced urinary levels). Aluminium containing prosthetic implants may increase blood and urine aluminium (San Martín, Bauçà, et Martinez-Morillo 2022).
Co-exposure to one or more substances (occupationally)	N/A
Route(s) of exposure, task description	N/A
Physical activity, effort, ...	N/A
Frequency and duration of exposure	N/A

5.4 Sampling, collection and storage of biological specimens

Strict precautions must be taken during sampling, sample preservation, sample preparation, and analysis. As for other ubiquitous chemicals, the risk of external contamination indeed appears as an issue in aluminium determination.

Below are some recommendations to collect and preserve blood and urine samples for aluminium measurement. However, as a general rule, sampling material should ideally be provided by laboratories that have previously checked their suitability for the analysis. Also, in the case of occupational biomonitoring exposure, samples should preferably be taken outside the workplace and after the worker has removed his work clothes and taken a shower. It is also advisable to enquire in advance, with the laboratory carrying out the analysis, about the pre-analytical conditions required, e.g.: storage and transportation requirements (SFMT 2016).

[37] https://www.inrs.fr/publications/bdd/biotox.html (accessed in April 2024)

The working group on biomarker of exposure at ANSES suggests the following recommendations to minimise contamination and ensure accurate results.

1-In the first instance, vials and consumables labelled "trace metal-free" should be used, and glass vials should be avoided.

2-If this is not the case, the equipment should be cleaned with 10% ultra-pure nitric acid (up to a maximum of 20%) and soaked overnight, then rinsed thoroughly with ultra-pure water.

3-In all cases, all consumables must be tested for aluminium contamination (blank run with concentrations of reagents and acids identical to those used in the samples). This should also be done after the cleaning step.

Urine samples

No recommendation on urine sampling time was made for the general population.

For aluminium exposed workers, it is generally recommended to carry out urine sampling at the end of the shift and after several shifts (Klotz et al. 2019), as reference health values were produced using such samples, which inform on both aluminium body burden and aluminium exposure during the last days (see chapter 4.8.1.1). Sampling has also been recommended at the beginning of the week, before the start of the day's work shift (WHO 1996; Biotox[38]).

In order to avoid the risk of contamination, samples should be collected at home, using the materials provided by the laboratory or otherwise it must be collected outside the workplace, ideally after showering or at least after washing hands.

As indicated above, materials used for sampling should be those recommended by the laboratory in charge of the analysis, which should have previously verified that they were free of aluminium. If not, precautions must be taken to avoid aluminium contamination of the material used (wash and soak overnight with 10% nitric acid, then rinse with ultrapure water and perform a blank test) (WHO 1996; Labat 2010; San Martín, Bauçà, et Martinez-Morillo 2022).

Blood samples

There is no recommendation on blood sampling time.

For workers, blood sampling is also recommended at the end of a shift after several shifts (WHO 1996; Biotox[39]). Whereas, for the general population, there is no recommendation on blood sampling time.

Most blood sampling tubes and tools are made of rubber containing aluminium silicate. For this reason, the collection of blood in specific vacuum tubes for trace elements is recommended. The use of separating gel vacuum tubes is permitted, but, as mentioned previously, the use of glass tubes is not.

As precedently indicated, materials used for sampling should be those recommended by the laboratory in charge of the analysis, which should have previously verified that they were free of aluminium otherwise, strict precautions must be taken (WHO 1996; Labat 2010; San Martín, Bauçà, and Martinez-Morillo 2022).

For serum measurements, after the blood has been collected and coagulated, it is centrifugated in a closed container (to avoid contamination or evaporation) for 10 minutes at 1,000-1,200 g. Serum is kept in suitable sealed polypropylene or polystyrene tubes for less than 14 days at 4°C (refrigerated) or is frozen at -20°C for prolonged preservation before analysis (WHO 1996; Labat 2010; San Martín, Bauçà, et Martinez-Morillo 2022).

5.5 Choice of the biomarker of exposure and the sampling time

Biomarker of exposure

When selecting an exposure biomarker, several criteria are generally taken into account: the association between concentrations of the potential biomarker and health effects and/or external concentrations or doses, the elimination half-life (important to decide on the sampling time), the specificity of the biomarker, the intra-individual and/or inter-individual variability of the association between exposure and the biomarker (in terms of

[38] https://www.inrs.fr/publications/bdd/biotox.html (accessed in April 2024)
[39] https://www.inrs.fr/publications/bdd/biotox.html (accessed in April 2024)

co-exposures, pathologies or predispositions), the sampling conditions and, the availability of analytical methods (ANSES, to be released) .

As shown in Table VII.63, urinary aluminium appears to be the biomarker of exposure of choice:

- there is sufficient evidence of a positive association between urinary aluminium level and the risk of health effects, with identified NOAEL and LOAEL in humans (see section 4.8.1.1);
- urine aluminium is a biomarker of greater sensitivity compared to serum aluminium when external exposure changes are minimal;
- in individuals with normal renal function, inter- and intra-individual variabilities are limited, when aluminium levels are adjusted on urine creatinine concentration, specific gravity or osmolality.
- Furthermore, urine sampling is non-invasive and analytical methods are available to conduct the analysis (see above section).

Sampling time

For the general population, generally exposed to aluminium on a chronic and not very variable basis, and apart from specific acute exposure, the time of sampling is uncritical. However, for good reproducibility and to avoid contamination by this ubiquitous compound, samples should be taken in the morning upon waking or after showering.

For workers, and from a practical point of view, the studies making it possible to characterise the association between urine aluminium concentration and the effects on health, and to identify a NOAEL and a LOAEL, have used urinary post-shifts sample (see section 4.8.1.1). As a consequence, the sampling time recommended for the biomonitoring of occupational aluminium exposure is the end of a shift, after several shifts.

In conclusion, urinary aluminium is selected as the relevant BME for biological monitoring of the general population and of the occupational exposure to aluminium, based on an analysis of the advantages and disadvantages of the various BMEs identified. In the case of impaired renal function, urinary aluminium cannot be used as BME because this pathological condition affects the interpretation of biomonitoring results.

6 Overview of existing TRV

6.1 Existing toxicological reference values for the general population

In 2006, US EPA has derived a provisional oral reference dose (pRfD) and a provisional inhalation reference concentration (pRfC) for the general population of 1 mg/Al/kg bw per day and 5 µg/m³ (respirable fraction). The oral point of departure (PoD) was obtained from a study in mice (Golub et al. 1995) where a LOAEL of 100 mg/kg bw per day[40] was considered for neurotoxicity in the offspring exposed in utero from conception through weaning. US EPA considered that increased grip strength has unclear toxicological significance, and increased grip strength and foot splay distance, did not persist after 2 weeks of no further exposure. An uncertainty factor (UF) of 3 was applied for using a LOAEL instead of a NOAEL (UF_L), a default UF_A of 10 for interspecies differences and an UF_H of 3 for interindividual differences as the effects were observed in a sensitive sub-group. This resulted in a pRfD of 1mg/kg bw per day (U.S. EPA 2006).

The pRfC derivation was based on human observations in a cohort of aluminium workers (Hosovski et al. 1990). Workers were exposed at concentrations of 4.6-11.5 mg Al/m³ (time-weighted average) for 12 years. US EPA did consider 4.6 mg Al/m³ as a LOAEC for psychomotor and cognitive impairment. An adjustment to continuous exposure duration was done considering respiratory rates of 20 m³ per day in the general population and 10 m³ per workshift in workers and continuous exposure in the general population instead of exposure for 5 days a week in workers resulting in a $LOAEC_{ADJ}$ of 1.64 mg Al/m³. The pRfC was obtained after application of an UF_H of 10 (although the key study is an epidemiological study), an UF_L of 10 for the use of a LOAEC instead of a NOAEC (usually a default value of 3 would have been used) and an UF_D of 3 for the lack of inhalation studies leading to an overall UF of 300. Thus, the calculated pRfC was of 5.5 µg/m³ rounded to 5 µg/m³. The use of default UF_H of 10 is not justified by US EPA and raises questions as the key study is an epidemiological study.

ATSDR (2008) has recommended a minimal risk level (MRL) of 1 mg/kg bw/day for chronic exposure. This MRL was derived from a study in mice (Golub et al. 2000) where mice were treated from conception to 2 years of age. Neurological effects were described as decreased forelimb and hindlimb strength leading to a LOAEL of 100 mg Al/kg bw/day according to ATSDR. This LOAEL was divided by an UF of 300 corresponding to an UF_A and UF_H (interspecies and interindividual variability) both set at 10 by default, an UF_L of 3 for using a LOAEL instead of a NOAEL. A modifying factor of 0.3 was also applied, considering that aluminium lactate (aluminium compound used for treatment) has higher bioavailability than aluminium supposedly retrieved in food and drinking diet. This led to a MRL of 10 µg/kg bw per day.

EFSA (2008) derived a tolerable weekly intake health-based guidance value (TWI) of 1 mg Al/kg bw per week (corresponding to 0.14 mg/kg bw per day). Two PoD were considered to derive this TWI. From the study by Golub et al. (1995) (mice treated from conception and through weaning), EFSA retained a LOAEL of 50 mg Al/kg bw per day based on grip strength reduction. In a second study in mice exposed from conception to 35 days old (Golub et Germann 2001), EFSA identified a LOAEL of 50 mg Al/kg bw per day for reduced body weight gain (only observed at 11 weeks) and a NOAEL of 10 mgAl/kg bw per day. Considering an UF of 300 applied to the LOAEL from the first study (UF_A and UF_H of 10, UF_L of 3) and an UF of 100 applied to the NOAEL in the second study (UF_A and UF_H of 10), EFSA derived two toxicological reference values of respectively 167 µgAl/kg bw per day and 100 µgAl/kg bw per day, that is 1.2 and 0.7 mgAl/kg bw per week which was averaged to 1 mgAl/kg bw per week.

In 2012, JEFCA considered a neurodevelopmental study in rats, (Poirier et al. 2011) to derive a provisional TWI (PTWI). Rats were treated from gestational day 6 to 364 days old through drinking water. As a result, water consumption has a significant impact on the aluminium citrate dose, which varies during the different life stages of the rats (e.g., during gestation, the dose was 10-14 below the target dose, up to 50% above during lactation, 30% below during weaning and then 15-45% above for the remainder of the study). Decrease in hindlimb and forelimb grip strength was observed alongside renal effects at 100 mg Al/kg bw per day. A NOAEL of 30 mg Al/kg bw per day was proposed by the authors of the study. As the effects on grip strength were more pronounced in young animals, exposure in utero or during lactation is likely more significant than exposure during later stages when the exposure dose decreased due to reduced drinking water consumption. JECFA also considered that the aluminium

[40] LOAEL of 155 mg/kg bw per day in Toxicological profile because of different methodologies to convert the dose in ppm

compound used, aluminium citrate, is more bioavailable than other compounds. Therefore, a NOAEL of 30 mg/kg body weight per day was assumed. Based on the evidence, an UF of 100 was used for interspecies and interindividual differences. No other UF was judged necessary. The derived toxicological reference value was 300 µg Al/kg bw per day approximatively corresponding to a PTWI of 2 mg Al/kg bw per week (corresponding to 0.29 mg/kg bw per day).

Table VII.65: Summary table of existing TRV

Toxicological Reference Value	Organism	US EPA	US EPA	ATSDR	EFSA	JECFA
	Year	2006	2006	2008	2008	2012
	Name	p-RfD	p-RfC	MRL	TWI	PTWI
	Value	1 mg/kg bw/d	5 µg/m³	1 mg/kg bw/d	1 mg/kg bw/ week corresponding to 0.14 mg/kg bw per day	2 mg/kg bw/ week corresponding to 0.29 mg/kg bw per day
Critical effect		Neuromuscular effects (decreased forelimb grip strength)	Psychomotor and cognitive impairment	Neuromuscular effects (decreased forelimb and hindlimb strength)	Neuromuscular effects (grip strength reduction and reduced body weight gain)	Renal effects (hydronephrosis, urethral dilatation, obstruction and/or presence of calculi) and Neuromuscular effects (decreased hindlimb and forelimb grip strength)
Key study	Reference	Golub et al. (1995)	Hosovski et al. (1990)	Golub et al. (2000)	1) Golub et al. (1995) 2) Golub & Germann 2001	Poirier et al. (2011)
	Study population or specie	Mice (SW)	Human, workers	Mice (SW)	1) and 2) Mice (SW)	Rats (SD)
	Exposure (duration, route)	From conception to weaning, diet	12 years, occupational	From conception to 2 years, diet	1) From conception to weaning, diet. 2) Conception to 35 days age, diet	From gestational day 6 to 364 days, drinking water
Point of departure (PoD)**		100 mg/kg bw/d (LOAEL)	4.6 mg Al/m³ (LOAEC)	100 mg/kg bw/d (LOAEL)	1) LOAEL: 50 mg/kg bw/d 2) LOAEL: 50 mg/kg bw/d NOAEL: 10 mg/kg bw/d	LOAEL: 100 mg/kg bw/d NOAEL: 30 mg/kg bw/d
Temporal adjustment		NA	1.64 mg/m³ ($LOAEC_{ADJ}$) (10 m³/20 m³ and 5 days/7 days)	NA	NA	NA
Allometric scalling		NA	NA	NA	NA	NA
Uncertainty factor (UF)		100 (UF_A: 10; UF_H: 3; UF_L: 3)	300 (UF_H: 10; UF_L: 10; UF_D: 3)	300 (UF_A: 10; UF_H: 10; UF_L: 3), modifying factor of 0.3 for bioavailability difference	1) 300 (UF_A: 10; UF_H: 10; UF_L: 3) 2) 100 (UF_A: 10; UF_H: 10)	100 (UF_A: 10; UF_H: 10)
Confidence level		Low	Low-Medium	NA	NA	NA

NA: not applicable

6.2 Human biomonitoring equivalent concentrations

Based on the PTWI derived by JECFA (2012), a biomonitoring equivalent concentration has been derived using a mass balance approach from the animal PoD (Poddalgoda et al. 2021). The authors also developed a kinetic model that has been used by the WG on biomarkers of exposure to derive a biomonitoring equivalent from the PTWI.

Mass balance approach

ANSES's WG on biomarkers of exposure considered deriving a biomonitoring equivalent (BE) from the oral TRV and not its related animal PoD. The equation used to derive a BE directly from the oral TRV is as followed:

$$BE = \frac{(PTWI \div 7) * F_{UE}}{V_{24}} = 117.1\ \mu g/L$$

Where F_{UE} is the urinary excretion fraction of aluminium citrate (0.0082) and V_{24} is the daily urinary volume (0.02 L/kg bw per day).

The BE value derived by Poddalgoda et al. (2021) from the animal PoD was of 123 µg/L which is equivalent to the BE obtained from the oral TRV.

Kinetic model

The three-compartment model developed by Poddalgoda et al. (2021) (see section 4.2) has been used (R software) to derive aluminium urinary excretion. A daily urinary volume of 0.02 L/kg bw per day is considered for a 70 kg bw adult. Parameters for aluminium citrate are used in the model as it is the most bioavailable compound.
A BE of 107.6 µg/L is derived from the PTWI of 2 mg/kg bw per week for adults.

Both approaches give similar results (107.6 - 117.1 µg/L).

7 Derivation of HBM-GV for aluminium and its compounds for the general population

In order to assess the risk to human health based on Aligned Biomonitoring Studies within PARC , a human biomonitoring guidance value is required for aluminium and its inorganic compounds.

7.1 Choice of the critical effect

The main systemic effect reported from epidemiological studies with workers occupationally exposed to aluminium is cognitive impairment observed as decreased neurobehavioral performance compared to unexposed individuals. Several epidemiological studies in the general population have investigated association between aluminium exposure and neurobehavioral performance. However, only aluminium levels in drinking water were measured and urinary aluminium was not assessed.

Thus, the cognitive impairment in exposed workers is selected as the critical effect in relation to urinary levels of aluminium.

7.2 Choice of the key study and identification of point of departure (PoD)

No human study conducted on the general population was identified from the literature search.

Human occupational data establishing a correlation between urinary aluminium concentrations and cognitive effects have been documented, providing the foundation for determining NOAEL and LOAEL. The convergence of evidence from two longitudinal studies, focusing on distinct cohorts of workers, delineates a NOAEL of 38 µg/g creatinine (post-shift). This was derived from an investigation involving aluminium welders in the car-body construction industry (98 workers and 50 controls) conducted by Buchta et al. in 2003 and subsequently affirmed by Kiesswetter et al. (2009). Furthermore, a LOAEL of 97 µg/g creatinine (post-shift) was established through research involving workers in the train and truck construction industry (44 workers and 37 controls), as demonstrated by Buchta et al. (2005) and confirmed by Kiesswetter et al. (2007).

Contrastingly, the longitudinal study conducted by Letzel et al. (2000) in an aluminium powder plant did not identify any discernible effects on the cognitive performance of workers compared to controls (32 workers and 30 controls on the first examination, 21 workers and 15 controls on the second one). Although plasma aluminium concentration among workers was comparable to that observed by Buchta et al. (2003), the urinary concentration was notably higher, 87.6 µg/g creatinine.

The lowest observed LOAEL were of 41.8 µg/g creatinine post-shift (Guo et al. 1999) and 40.1 µg/g creatinine pre-shift (He et al., 2003). In Guo et al. (1999), cognitive performance was impaired inconsistently across age groups, while in He et al. (2003), exposed workers had significantly better reaction times than controls, despite significantly lower scores on digital symbol test and pursuit aiming test.

In the absence of epidemiological studies in the general population, the NOAEL of 38 µg/g creatinine post-shift from the longitudinal study of Buchta et al. (2003) and Kiesswetter et al. (2009) is retained as the point of departure to derive the HBM-GV for the general population.

7.3 Application of uncertainty factors

The HBM-GV for the general population was calculated using uncertainty factors (UF) described below:

- inter-species uncertainty factor (UF_A): 1 because the internal TRV is based on human data;
- inter-individual uncertainty factor (UF_H): 2 considering the ratio between the default inter-individual uncertainty factors of 10 and 5 for the general population and workers, respectively;
- sub-chronic to chronic uncertainty factor (UF_S): 1 because individuals were chronically exposed ;
- use of LOAEL uncertainty factor (UF_L): 1. The retained PoD is a NOAEL, no additional factor is needed;
- quality of the database uncertainty factor (UF_D): 1. Aluminium is a data-rich substance, and numerous studies were published on the potential effects of aluminium.

The overall UF for deriving the HBM-GV for the general population is 2.

7.4 HBM-GV for the general population

The HBM-GV of 20 µg/g creatinine is proposed for the general population based on the NOAEL of 38 µg/g creatinine, rounded to 40 µg/g creatinine and an UF of 2.

Furthermore, by comparing the BEs derived from JECFA's oral TRV using the mass balance approach (117.1 µg/L) or the 3-compartment kinetic model (107.6 µg/L) (see section 6.2), with the NOAEL of 47.6 µg/L retained from the epidemiological studies by Buchta et al. (2003) and Kiesswetter et al. (2009) (value provided in the article with the creatinine-adjusted concentration of 37.87 µg/g creatinine), we note that the BEs are higher, and for this reason they were not retained. The proposed, more conservative, HBM-GV, based on a PoD from a human study (workers), with an uncertainty factor of 2 to account for inter-individual variability, is therefore selected. This approach also aligns with the HBM-GV derivation methodology (see chapter 2).

Table VII.66: HBM-GV for the general population summary

Biomarker of exposure		**Urinary aluminium**
Toxicological Reference Value	**Name**	**HBM-GV**
	Value	**20 µg/g creatinine; morning**
Target population		General population
Applied methodology		Relation internal dose/health effects
Critical effect		Cognitive and motor effects
Key study	**Reference**	Buchta et al. (2003) ; Kiesswetter et al. (2009)
	Species	Human
	Exposure (duration, route)	4.7 years ± 1.6 Inhalation, ingestion, cutaneous at workplace
PoD		38 µg/g creatinine (NOAEL)
Adjustment		NA
Uncertainty factor (UF)		2 (UF_A: 1; UF_H: 2; UF_L: 1; UF_S: 1; UF_D: 1)

8 Overview of existing HBM-GVs for workers

In 2019, the MAK commission (German Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area) (Klotz et al. 2019) established a biological tolerance value (BAT) of 50 µg Al/g creatinine as measure in post-shift urine after several shifts. DFG considered an approach based on the relationship between internal dose and health effects. Based on epidemiological observations in workers occupationally exposed to aluminium, cognitive effects were considered as the critical effect corresponding to preclinical neurotoxic effects.

Based on two longitudinal studies, one conducted in train and truck construction industry (Mark Buchta et al. 2005; E. Kiesswetter et al. 2007) and the other one in automotive engineering (M. Buchta et al. 2003; Ernst Kiesswetter et al. 2009), the MAK commission identified a LOAEL of approximatively 100 µg/g creatinine (rounded from a median of 97 µg/g creatinine) and a NOAEL of 38 µg/g creatinine for aluminium concentration in post-shift urine after several shifts. Significant changes observed at the LOAEL were decreased performance in symbol-digit substitution test, attention-switching test and block design test.

In a second step, DFG analysed data from 12 epidemiological studies of neurocognitive effects in workers exposed to aluminium for the evaluation of the effect size of each study (Hosovski et al. 1990; Bast-Pettersen et al. 1994; Sjögren et al. 1996; Akila, Stollery, et Riihimäki 1999; Guo et al. 1999; Bast-Pettersen et al. 2000; V. Riihimäki et al. 2000; M. Buchta et al. 2003; He, Qiao, et Sheng 2003; Mark Buchta et al. 2005; E. Kiesswetter et al. 2007; Ernst Kiesswetter et al. 2009). The effect size estimated for each study was estimated as standardised mean differences (i.e., difference between the mean test results of the exposed group and the control group, divided by the variance in the control group).

This approach allowed to demonstrate that a higher effect size was observed in studies with a median urinary concentration higher than 50 µg/g creatinine in exposed workers, while those studies where median urine aluminium concentration in the exposed group was below 50 µg/g creatinine had effect sizes considered small by the MAK Commission. Thus, a BAT value of 50 µg/g creatinine was derived from a synopsis of the results of both the longitudinal studies by Buchta et al. (2003) and Kiesswetter et al. (2009), and of the analysis with the recommendation to measure urinary levels at the end of a shift after several shifts.

Table VII.67: Summary table of existing HBM-GV for workers

BME		**Urinary Aluminium**
Organisation		**DFG**
Year		**2019**
Toxicological Reference Value	**Name**	**BAT**
	Value	**50 µg/g creatinine; end of shift after several shifts**
Target population		**Workers**
Applied methodology		**Relation internal dose/health effects**
Critical effect		Cognitive and motor effects
Key study	**Reference**	Buchta et al. (2003); Kiesswetter et al. (2009) Analysis by the BAT Working Group
	Species	Human
	Exposure (duration, route)	4.7 years ± 1.6 Inhalation, ingestion, cutaneous at workplace
PoD		38 µg/g creatinine (NOAEL)
Adjustment		NA
Uncertainty factor (UF)		1 (UF_A: 1; UF_H: 1; UF_L: 1; UF_S: 1; UF_D: 1)

9 Derivation of HBM-GVs for workers

9.1 Choice of critical effect

Cognitive impairment in exposed workers was selected as the critical effect in relation to urinary levels of aluminium from epidemiological studies in workers in aluminium industries.

9.2 Choice of construction hypothesis

For most non-carcinogenic effects, it is generally considered by default and in the current state of knowledge that toxicity is only expressed above a certain dose threshold.

Thus, the HBM-GV for workers will be based on the cognitive impairment results from a dose-threshold mechanism.

9.3 Choice of key-study and point of departure

Human occupational data establishing a correlation between urinary aluminium concentrations and cognitive effects have been documented, providing the foundation for determining NOAEL and LOAEL. The convergence of evidence from two longitudinal studies, focusing on distinct cohorts of workers, delineates a NOAEL of 38 µg/g creatinine (post-shift). This was derived from an investigation involving aluminium welders in the car-body construction industry (98 workers and 50 controls) conducted by Buchta et al. (2003) and subsequently affirmed by Kiesswetter et al. (2009). Furthermore, a LOAEL of 97 µg/g creatinine (post-shift) was established by the ANSES Working Group from a longitudinal study involving workers in the train and truck construction industry (44 workers and 37 controls), as demonstrated by Buchta et al. (2005) and confirmed by Kiesswetter et al. (2007).

Contrastingly, the longitudinal study conducted by Letzel et al. (2000) did not identify any discernible effects on the cognitive performance of workers compared to controls (32 workers and 30 controls on the first examination, 21 workers and 15 controls on the second one). Although plasma aluminium concentration among workers was comparable to that observed by Buchta et al. (2003), the urinary concentration was notably higher at 87.6 µg/g creatinine.

The lowest observed LOAEL were 41.8 µg/g creatinine post-shift (Guo et al. 1999) and 40.1 µg/g creatinine pre-shift (He et al., 2003). In Guo et al. (1999), cognitive performance was impaired in an inconsistent manner across age groups, while in He et al. (2003), exposed workers had significantly better reaction times than controls despite significantly lower scores in the digital symbol test and pursuit aiming test.

Thus, the NOAEL of 38 µg/g creatinine post-shift from the longitudinal studies by Buchta et al. (2003) and Kiesswetter et al. (2009) is retained as the point of departure to derive the HBM-GV for workers.

9.4 Application of uncertainty factor

No additional uncertainty factor (UF) is considered necessary as the target population correspond to the studied population from the longitudinal study:

- inter-species uncertainty factor (UF_A): 1 because the NOAEL is based on human data;
- inter-individual uncertainty factor (UF_H): 1 because the key study corresponds to the target population;
- sub-chronic to chronic uncertainty factor (UF_S): 1 because individuals were chronically exposed;
- use of LOAEL uncertainty factor (UF_L): 1. A NOAEL is retained as PoD no additional factor is needed;
- quality of the database uncertainty factor (UF_D): 1. Aluminium is a data-rich substance and numerous studies were published on the potential effects of aluminium.

The overall UF for deriving the HBM-GV for workers is 1.

9.5 HBM-GV for workers

The HBM-GV for workers for aluminium is derived from the NOAEL of 38 µg/g creatinine with an uncertainty factor of 1, rounded to 40 µg/g creatinine.

Thus, an HBM-GV of 40 µg/g creatinine post-shift based on neurotoxicity is proposed for urinary aluminium.

Table VII.68: HBM-GV for workers summary

Biomarker of exposure		**Urinary aluminium**
Toxicological Reference Value	**Name**	**HBM-GV**
	Value	**40 µg/g creatinine; end of shift after several shifts**
Target population		**Workers**
Applied methodology		**Relation internal dose/health effects**
Critical effect		**Cognitive and motor effects**
Key study	**Reference**	**Buchta et al. (2003); Kiesswetter et al. (2009)**
	Specie	**Human**
	Exposure (duration, route)	**4.7 years ± 1.6** **Inhalation, ingestion, cutaneous at workplace**
PoD		**38 µg/g creatinine (NOAEL)**
Adjustment		**NA**
Uncertainty factor (UF)		**1** (UF_A: 1; UF_H: 1; UF_L: 1; UF_S: 1; UF_D: 1)

10 Confidence levels

Several criteria need to be taken into account when assigning a confidence level to the derived HBM-GVs of the general population and for workers:

- A wealth of data is available, and numerous animal and human epidemiological studies have made it possible to establish a toxicological profile for aluminium and its inorganic compounds.
- Urinary aluminium is a specific and sensitive biomarker of exposure to aluminium compounds. An association between urinary aluminium levels and health effects has been demonstrated, and analytical methods are available to perform the analyses.
- Although some epidemiological studies have revealed no neurological effects or have presented inconsistent findings, the selected longitudinal study was carried out in the automotive industry on welders exposed only to aluminium (with no exposure to other potentially neurotoxic substances) and on unexposed controls.
- No allometric or temporal adjustments were necessary.
- Only an uncertainty factor of 2 to account for inter-human variability was applied to calculate the HBM-GV for the general population, and no uncertainty factor was applied to the HBM-GV for workers.

Consequently, a medium-high level of confidence is attributed to these two HBM-GVs.

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Part VIII

Derivation of human biomonitoring guidance values (HBM-GVs) for **benzophenone-3** (BP-3, Oxybenzone, 2-hydroxy-4-methoxybenzophenone, HMB, CAS-No.: 131-57-7) for the general population

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Contributing Participants	Manca Ahačič (NIJZ), manca.ahacic@nijz.si Lucija Perharič (NIJZ), lucija.perharic@nijz.si Maria Tannous (ANSES)
Responsible author(s)	Antje Gerofke (UBA)
Co-authors	Petra Apel (UBA), petra.apel@uba.de
Internal reviewers	WP4 co-leaders
External reviewers	Experts from Danish NH, Belgian NH, French NH, Norwegian NH; UK NH Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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1 PU = Public
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Final Version	12.03.2025	Review of AD 4.3 by EAA	Update of 24h bodyweight-related urine volume for adults and recalculation of HBM-GVs for adults.

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Abstract

Under PARC, WP4, project 4.1.3.1.a, human biomonitoring guidance values (HBM-GVs) are derived for priority substances. These health-based HBM-GVs can be directly compared with HBM data and, as jointly agreed values under PARC, are a valuable tool for the harmonised assessment of health risks. The HBM-GVs are derived as needed for the general population and/or occupationally exposed adults.

The human biomonitoring guidance value for the general population (HBM-GV_{GenPop}) represents the concentration of a substance (or metabolite) in human biological material (mostly urine or blood, rarely other tissues) up to which, according to current knowledge - when considering individual substances - adverse health effects are not expected. An exceedance of this value should be a reason to investigate the causes and to minimise possible sources of exposure (Apel et al., 2020). In the current report, the derivation of HBM-GV_{GenPop}, both for children and adults, is presented and discussed for the UV-filter benzophenone-3 (BP-3) (CAS No.: 131-57-7), also named 2-hydroxy-4-methoxybenzophenone (HMB), or oxybenzone. Of the commercially available benzophenones, BP-3 is the most widely applied UV-filter in consumer products. Due to its widespread use, human exposure to BP-3 is common.

A provisional HBM-GV has already been derived under HBM4EU, but this had not been subject to a consultation procedure and so had not been jointly agreed within HBM4EU. For this reason, the derivation of the provisional value was reviewed and updated. In this update, reproductive toxicity remained the most sensitive effect for BP-3. For the derivation of the HBM-GV, urine volume was taken as reference instead of creatinine. The update of 24h bodyweight-related urine volume for adults (German HBM Commission, 2025, submitted) was considered for the calculation of HBM-GVs for adults.[41]

Health-based HBM-GV_{GenPop} were set to 226 µg BP-3/L urine for children and men and 194 µg BP-3/L urine for women.

Key Words

Human biomonitoring, benzophenone-3 (BP-3), UV-filter, human biomonitoring guidance values, HBM-GV, risk assessment, chemical policy

[41] By using the outdated 24h bodyweight-related urine volume for men and women of 0.02 L/(kg bw x d) for the calculation of HBM-GV, the resulting HBM-$GV_{GenPop\ (adults)}$ would have been 340 µg BP-3/L urine.

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Authors and Acknowledgements

Authors

Antje Gerofke, UBA, Germany

Petra Apel, UBA, Germany

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Background

Within the framework of PARC, health-based HBM guidance values (HBM-GVs) are established for priority substances as needed for the general population (HBM-GV_{GenPop}) and/or for workers (HBM-GV_{worker}) after their derivation approach underwent a consultation process by national experts nominated by PARC partners. The general methodological approach was published in 2020 (Apel et al., 2020).

In the current report, the derivation of HBM-GV_{GenPop}, both for children and adults, is presented and discussed for benzophenone-3 (BP-3) (CAS No.: 131-57-7). The derivation is based on the derivation of a provisional adult urine HBM-GV_{GenPop} from HBM4EU, with reference to creatinine-normalised urine (Woutersen et al., 2022; Rousselle et al., 2022) which could not be agreed at the time. In the update described here, reproductive toxicity remained the most sensitive effect for BP-3. A urine volume reference was selected here for the HBM-GV derivation. The update of 24h bodyweight-related urine volume for adults (German HBM Commission, 2025, submitted) was considered for the calculation of HBM-GVs for adults.

For the toxicological endpoints assessed, information was taken from the opinions of the Scientific Committee on Consumer Products (SCCP) (2006, 2008), the opinion of the Scientific Committee on Consumer Safety (SCCS) (2021), the registration dossier (ECHA dissemination, 2021), the review on BP-3 by Mustieles et al., 2023, as well as from further literature. The literature search for this report was completed on 22nd June 2023.

Results

Reproductive toxic effects of BP-3 were selected as critical effects (reduction of the number of spermatocytes per seminiferous tubule in offspring). Health-based HBM-GV_{GenPop} were set to 226 µg BP-3/L urine for children and men and 194 µg BP-3/L urine for women. [42]

The HBM-GV_{GenPop} were derived for the total (conjugated and free) BP-3 in urine since this is routinely measured in HBM studies.

[42] By using the outdated 24h bodyweight-related urine volume for men and women of 0.02 L/(kg bw x d) for the calculation of HBM-GV, the resulting HBM-$GV_{GenPop\ (adults)}$ would have been 340 µg BP-3/L urine.

Abbreviations and Acronyms

AF	Assessment Factor
BP-3	Benzophenone-3
bw	body weight
CAS	Chemical Abstracts Service (division of the American Chemical Society)
CLP	Classification, Labelling and Packaging of substances
d	day
D 5.8	Deliverable 5.8 within HBM4EU
D 5.11	Deliverable 5.11 within HBM4EU
DHB	2,4-dihydroxybenzophenone (BP-1)
DHMB	2,2'-dihydroxy-4-methoxybenzophenone (BP-8)
DMF	Dimethylformamide
DNEL	Derived No-Effect Level
EC	European Commission
EC_{50}	Half-maximal effective concentration
ECHA	European Chemicals Agency
FSH	Follicle-Stimulating Hormone
ESB	German Environmental Specimen Bank
F1	First filial generation
F2	Second filial generation
FDA	US Food and Drug Administration
Fue	Urinary excretion factor
GD	Gestational Day
GLP	Good Laboratory Practice
GM	Geometric Mean
h	hour
HBM	Human Biomonitoring
HBM4EU	European Human Biomonitoring Initiative
HBM-GV	Human biomonitoring guidance value
HMB	2-hydroxy-4-methoxybenzophenone (BP-3)
HPLC	High Performance Liquid Chromatography
IC_{50}	Half-maximal inhibitory concentration
kg	kilogramme

LC50	Lethal Concentration 50 %
LC/MS	Liquid Chromatography/Mass Spectrometry
LD	Lactation Day
LD50	Lethal Dose 50 %
LH	Luteinizing Hormone
LLoQ	Lowest Limit of Quantification
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
Log P_{OW}	Decadic logarithm of the Octanol/Water Partition Coefficient
LOQ	Limit of Quantification
MoS	Margin of Safety
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
OECD	Organisation for Economic Co-operation and Development
OX	Oxybenzone
O/W	Oil-in-Water
P0, P1, P2	Parental generation 0, Parental generation 1, Parental generation 2
PBPK/PBTK	Physiologically Based PharmacoKinetic (ToxicoKinetic) modelling
PBT/ vPvB	Persistent, Bio-accumulative and Toxic/ very Persistent and very Bioaccumulative
PCPs	Personal Care Products
PND	Postnatal Day
POD	Point Of Departure
RAC	Risk Assessment Committee
RACB	Reproductive Assessment by Continuous Breeding
REACH	Registration, Evaluation and Authorisation of Chemicals
RHR	Rat to Human Ratio
SCCS	Scientific Committee on Consumer Safety
SCCP	Scientific Committee on Consumer Products
SCCNFP	Scientific Committee on Consumer Products and Non-food Products intended for Consumers
SCOEL	Scientific Committee on Occupational Exposure Limits
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SED	Systemic Exposure Dose

SVHC	Substances of Very High Concern
TAF	Total Assessment Factor
TDI	Tolerable Daily Intake
TGB	Thyroglobulin
THB	2,3,4-trihydroxybenzophenone
TRV	Toxicological (Toxicity) Reference Value
TSH	Thyroid-Stimulating Hormone
TT	Total Testosterone
T3	Triiodothyronine, thyroid hormone
T4	Thyroxine, thyroid hormone
UV	Ultraviolet
WBC	White Blood Cells
W/O	Water-in-Oil
WHO	World Health Organisation

1 Introduction

In the current report, the derivation of human biomonitoring guidance values (HBM-GVs) for the general population (HBM-GV_{GenPop}) both for children and adults is presented and discussed for benzophenone-3 (BP-3) (CAS No.: 131-57-7), also named 2-hydroxy-4-methoxybenzophenone (HMB), and referred to as "oxybenzone". Throughout this dossier BP-3 is used for the compound.

Benzophenones are lipophilic phenols chemically synthesised for their UVA and UVB absorbing and dissipating properties. Of the commercially available benzophenones, BP-3 is the most widely applied UV-filter in sunscreens and cosmetics (Mustieles et al., 2023). Due to its widespread use, human exposures to BP-3 are common. In the HBM4EU Aligned Studies, BP-3 was detected in relatively low levels in all countries investigated.

Benzophenone-1 (BP-1) (CAS No.: 131-56-6), is the major metabolite of BP-3 both in rodents and humans (Mutlu et al., 2020). In the HBM4EU Aligned Studies, BP-3 was detected in relatively low levels in all countries investigated. However, total (conjugated and free) BP-3 concentration is commonly measured in urine as a biomarker for internal exposure.

The derivation of the HBM-GV_{GenPop} is based on the deliverable D 5.11 from HBM4EU (Woutersen et al., 2022) where a provisional HBM-GV_{GenPop} was derived for adults (Woutersen et al., 2022; Rousselle et al., 2022).

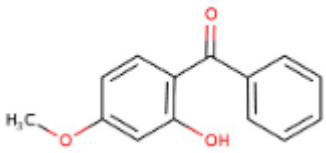
For the toxicological endpoints assessed, information was taken from the registration dossier (ECHA dissemination, 2021), the opinions of the Scientific Committee on Consumer Products (SCCP) (2006, 2008), the opinion of the Scientific Committee on Consumer Safety (SCCS, 2021), as well as from literature (whereby mainly referring to the recent systematic review by Mustieles et al. (2023)). The literature search of this report was completed on 22nd June 2023.

The state of epidemiological knowledge based on human exposure to BP-3, on whether and how it might cause human health effects is not sufficient to derive HBM-GVs directly based on human data. Thus, the derivation of HBM-GVs relies on toxicity findings from animal studies (i.e., oral rat studies) with a conversion to human urinary metabolite levels using human data by Hayden et al. 1997 and Sarveiya et al. 2004. Reproductive toxic effects of BP-3 were selected as critical effects. HBM-GV_{GenPop} for BP-3 were derived in relation to urine volume, without adjustment for creatinine.

2 General information and physical-chemical properties of BP-3

BP-3 is an off-white to light yellow powder with a melting point of 62°C to 65°C, absorbs ultraviolet (UV) A (320 to 400 nm) and UVB (290 to 320 nm) light and is photostable (SCCS, 2021).

Table VIII.1: General information on benzophenone-3

Substance	Benzophenone-3 (BP-3)
Common name	Benzophenone-3 (BP-3)
Trade names	Benzophenone-3 (BP3); Escalol 567, Eversorb 11, Speedblock UV-9,
IUPAC names*	2-benzoyl-5-methoxyphenol; 2-Hydroxy-4-methoxybenzophenone (HMB)
EC-name	Oxybenzone
CAS-number*	131-57-7
EC-number*	205-031-5
Molecular formula*	$C_{14}H_{12}O_3$
Molecular mass*	228.24 g/mol
Structural formula	 **Figure VIII.1: Structural formula of BP-3 (Source: ECHA CHEM Database)**

*: Data taken from the registration dossier of benzophenone-3 (ECHA Dissemination, 2021)

Table VIII.2: Physical-chemical properties of benzophenone-3

Substance	Benzophenone-3*
Vapour pressure (at 20 °C)*	0.0005 Pa
Vapour pressure (at 25°C)	0.0011 Pa
Melting/ freezing point* (1,013 hPa)	62.9 °C
Boiling point (at 99.86kPa)	>300 °C
Relative density (at 20 °C)*	1.43
Log Pow (at 40 °C)*	3.45
Water solubility (at 25 °C)*	6 mg/L
Physical state at 20 °C, 1,013 hPa*	solid
Colour	yellowish

*: Data taken from the registration dossier of benzophenone-3 (ECHA Dissemination, 2021)

3 Occurrence and use of BP-3

BP-3 is a UV-filter widely applied in a variety of consumer products, principally sunscreens and other cosmetic products (Mustieles et al., 2023). BP-3 can also be added to paints and coatings as a UV-stabiliser (DEPA, 2015). Mustieles et al. (2023) report that benzophenone and its derivatives naturally occur in flowering plants such as mango and muscat grape (Zhang et al., 2019), but are synthesized for commercial use. BP-3 is manufactured in/or imported to the European Economic Area in 1000-10000 tonnes per year; it is used by consumers, by professional workers, in formulations or re-packaging and at industrial sites (ECHA dissemination, 2021).

Release to the environment is likely to occur from industrial as well as household uses (e.g. machine wash detergents, personal care products, paints and coating, fragrances and air fresheners) (ECHA dissemination, 2021).

Use of personal care products (PCPs) likely constitutes the major human exposure source (Mustieles et al., 2023). As reported in SCCS (2021), recent studies indicate that there might be other sources for BP-3 besides sunscreen and cosmetics. Other potential sources of BP-3 are listed in the review by Mustieles et al. (2023) and include as primary source e.g. textiles (Li and Kannan, 2018; Xue et al., 2017), as secondary sources (contaminated by the primary ones): indoor dust (Ao et al., 2018; Shin et al., 2020) and air (Dodson et al., 2019; Wan et al., 2015), drinking tap water (da Silva et al., 2015; Díaz-Cruz et al., 2012), seafood (Cunha et al., 2018) and potentially, plastic-packaged foods and beverages (Dodson et al., 2020; Kim et al., 2016). The relative contribution of non-PCP sources to human exposure remains unclear, however, Mustieles et al. (2023) conclude that these additional dietary and non-dietary sources may help to explain the sustained BP-3 exposure observed in all population groups irrespective of the season and sunscreen use (Frederiksen et al., 2013a; Frederiksen et al., 2013b). The extent to which BP-1 (the major metabolite of BP-3) is directly used in PCPs is unknown (Mustieles et al., 2023).

4 Legal framework

SCCS was mandated by the Commission to review the safety of BP-3, it was due to the ED concern as explained in the background of the SCCS opinion: "On 7 November 2018, the Commission adopted a review of Regulation (EC) No 1223/2009 on cosmetic products ('Cosmetics Regulation') regarding substances with endocrine disrupting properties. The review concluded that the Cosmetics Regulation provides the adequate tools to regulate the use of cosmetic substances that present a potential risk for human health, including when displaying ED properties. The Cosmetics Regulation does not have specific provisions on EDs. However, it provides a regulatory framework with a view to ensuring a high level of protection of human health. Environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 ('REACH Regulation'). In the review, the Commission commits to establishing a priority list of potential EDs not already covered by bans or restrictions in the Cosmetics Regulation for their subsequent safety assessment. A priority list of 28 potential EDs in cosmetics was consolidated in early 2019 based on input provided through a stakeholder consultation. The Commission then organised a public call for data2 from 16 May 2019 – 15 October 2019 on 14 of the 28 substances (to be treated with higher priority) in order to be able to prepare the safety assessment of these substances. Benzophenone-3 is one of the above-mentioned 14 substances for which the call for data took place."

On the basis of safety assessment, and considering the concerns related to potential endocrine disrupting properties of benzophenone-3 (BP-3), the SCCS (2021) concluded that:

1. The use of BP-3 as a UV filter in the following sunscreen products is safe for the consumer up to a maximum concentration of 2.2% in body creams, in propellant sprays and in pump sprays, provided that there is no additional use of BP-3 at 0.5% in the same formulation for protecting the cosmetic formulation,

2. Where BP-3 is also used at 0.5% in the same formulation, the levels of BP-3 used as UV filter should not exceed 1.7% in body creams, in propellant sprays and in pump sprays.

3. The use of BP-3 as a UV-filter up to a maximum concentration of 6% in face cream, hand cream, and lipsticks is safe for the consumer.

4. The use of BP-3 up to 0.5% in cosmetic products to protect the cosmetic formulation is safe for the consumer.

The Regulation (EC) No 1223/2009 of the European Parliament and of the Council as regards the use of certain UV filters in cosmetic products was amended accordingly (COMMISSION REGULATION (EU) 2022/1176 of 7 July 2022)

Classification provided by companies

According to the classification provided by companies to ECHA in CLP notifications this substance is toxic to aquatic life with long lasting effects, is very toxic to aquatic life, causes serious eye and skin irritation and may cause respiratory irritation.

Benzophenone-3 has no PBT/vPvB properties (ECHA Dissemination, 2021).

5 Exposure of the general population

BP-3 has been detected in human urine, serum and breast milk (SCCS, 2021). Santamaria et al. (2020) could confirm that BP-3 can cross the placental barrier in mice.

Within HBM4EU levels of benzophenone UV-filters in urine samples were determined in six studies (HBM4EU Aligned Studies) from 2014-2018. An overview of the study results is given in Table VIII.3 (taken from HBM4EU D5.11, i.e., Woutersen et al., 2022).

Table VIII.3: Overview of the outcome of the BP-3 measurements in the HBM4EU Aligned Studies in µg/g creatinine (taken from HBM4EU D5.11 (i.e., Woutersen et al., 2022))

Country	Sampling period	N	Age range	Sample	% above LOD/LOQ	P05	P10	P25	P50	P75	P90	P95
Luxembourg	2016-2018	210	25-39	Spot	99.51%	0.13	0.17	0.44	0.94	2.64	10.21	18.97
Germany	2014-2017	180	20-29	24 hr	30%	<LOQ	<LOQ	<LOQ	<LOQ	3.00	11.25	27.52
Not specified	2015-2017	56	12-18	Morning	30.36%	<LOQ	<LOQ	<LOQ	<LOQ	3.25	7.10	21.62
Norway	2016-2017	181	12-14	Spot	100%	0.66	0.80	1.55	3.50	8.25	28.84	55.92
Poland	2017	249	12-14	Spot	100%	0.59	0.76	1.40	3.68	9.52	34.97	67.22
Sweden	2016-2017	300	12-17	Spot	94.33%	<LOD	0.11	0.29	0.88	2.64	11.31	33.87
Spain	2017-2018	299	13-17	Spot	99%	0.46	0.65	1.45	3.01	7.20	30.25	68.83

BP-3 was detected in relatively low levels in all countries investigated. However, there were differences in LOQ/LOD which must be kept in mind.

Calafat et al. (2008) detected BP-3 in 96.8% of the tested urine samples from the general U.S. population, with mean concentrations of 22.9 ng/mL (22.2 µg/g creatinine). Concentrations were significantly higher for females than for males, regardless of age.

Mustieles et al. (2023) performed a meta-analysis of human biomonitoring studies investigating BP-3 exposure and found in sub-group analysis by geographical region, that the overall pooled average concentration of BP-3 was highest for North American studies (22.78 ng/mL ≈ µg/g creatinine). Average pooled concentrations for Europe and Asia were, respectively, about 10-times (2.88) and 20-times (1.25) lower ($p < 0.0001$). Reported 95-percentiles for urinary BP-3 concentrations ranged from 0.68 to 6740 ng/mL and thus varied more between studies than medians/geometric means. Studies with the lowest reported 95-percentile were mainly from China and those with the highest were mainly from Northern American women in their mid-thirties.

It can be assumed that exposure peaks are the result of large-scale dermal application, but that the background exposure of the population is also oral (hand-mouth contacts, lipstick application) and possibly also inhalational (use of sprays containing BP-3) (see also section 3). Mustieles et al. (2023) conclude that dermal exposure through sunscreen and cosmetics is linked to peak-exposures, whereas a continuous low-dose exposure is linked to food, textiles, indoor air and dust (see also section 3).

6 Toxicological profile of BP-3

The toxicological database for BP-3, especially regarding animal studies, is very extensive; only the most relevant toxicity data are used for the derivation of tolerable doses and the most relevant studies providing kinetic data necessary for the proposal of HBM-GVs will be reported here in more detail.

For the toxicological endpoints assessed, information is taken from the registration dossier (ECHA dissemination, 2021), the opinions of the Scientific Committee on Consumer Products (SCCP) (2006, 2008), the opinion of the Scientific Committee on Consumer Safety (SCCS, 2021), as well as from literature (whereby mainly referring to the recent systematic review by Mustieles et al. (2023)). The literature review of this report was completed on 22nd June 2023.

In Mustieles et al. (2023), references that belong to the following categories were selected: exposure sources and predictors (35 studies); human exposure (125 studies); human exposure-health associations (67 studies); human interventions (toxicokinetics) (9 studies); *in vitro* bioassays (55 studies); *in vivo* studies on rodents (25 studies).

Main findings are reported in this dossier, details from relevant studies are summarised in the Appendix.

6.1 Toxicokinetics of benzophenone-3

6.1.1 Human data

A large body of evidence based on human data implies rapid absorption and systemic exposure after oral and dermal administration of BP-3 (e.g., Gonzalez et al., 2006; Hayden et al., 1997; Janjua et al., 2004; 2008). Also, inhalation/ingestion of dust has to be assumed, but despite notable concentrations of BP-3 in indoor air and dust samples, this only accounted for approximately 5% of the total intake of BP-3 (Wan et al., 2015).

After absorption, BP-3 is metabolized into numerous metabolites including BP-1, BP-2, BP-8, and 4-HBP, with BP-1 being the major metabolite of BP-3 (in humans as in rodents) (Gao et al., 2015; Mustieles et al., 2023). BP-3 and its metabolites are also conjugated (see figure 2 below). The main metabolites of BP-3 in humans are presented in Table VIII.4.

Table VIII.4: Information on main BP-3 metabolites in humans (modified according to Gao et al., 2015)[43]

Commercial name	Chemical name	Chemical abbreviations	Molecular weight	CAS no.
BP-3[44]	2-hydroxy-4-methoxy benzophenone (**HMB**)	2-OH-4MeO-BP	228.24	131-57-7
4-HBP[45]	4-hydroxy benzophenone	4-OH-BP	198.22	1137-42-4
BP-1	2,4-dihydroxy benzophenone (**DHB**)	2,4-diOH-BP	214.22	131-56-6
BP-2	2,2' ,4,4' -tetrahydroxy benzophenone	2,2',4,4'-tetraOH-BP	246.22	131-55-5
BP-8	2,2' -dihydroxy-4-methoxy benzophenone (**DHMB**)	2,2'-diOH-4-MeO-BP	244.24	131-53-3

In their review, Mustieles et al. (2023) report on nine human dermal toxicokinetic studies in relation to BP-3 and its metabolite BP-1 (i.e., Matta et al., 2019; Matta et al., 2020; Janjua et al., 2008, 2007, 2004; Gonzalez et al., 2006; Gustavsson et al., 2002; Sarveiya et al., 2004; Hayden et al., 1997). Study summaries and further information are

[43] 2,3,4-trihydroxybenzophenone (THB) is a minor metabolite

[44] Total (free plus conjugated) BP-3 concentration is commonly measured in urine as biomarker for internal exposure.

[45] 4-HBP is also used as an industrial chemical on its own account, e.g. as anti-bacterial agent (see also https://doi.org/10.1533/9780857092939.2.97) and in (pharma) industry as intermediate to produce other chemicals, e.g. Clomifen

given in Table VIII.A1 of the Appendix. In summary, these reports have shown that BP-3 is absorbed through the skin, is systemically available, and is mainly excreted in the urine. Mustieles et al. (2023) remarked that "skin accumulated BP-3 acted as a reservoir".

Dermal studies from which quantitative estimates can be made are by Hayden et al. (1997) showing that after applying a commercially available sunscreen product with a BP-3 content of 6% (w/v) the actually absorbed portion of the applied amount of active ingredient was between 1 and 2% over a period of 10 hours, and by Sarveiya et al. (2004) showing that up to approximately 1% of the applied dose of BP-3 were detected in urine as BP-3 and its metabolites. The major metabolite identified by Sarveiya et al. (2004) was 2,4 dihydroxybenzophenone (DHB; BP-1), whereas 2,3,4-trihydroxybenzophenone (THB) was detected only in trace amounts.

Detection in breast milk

In milk samples from 83 donors from a human milk bank in Granada, Southern Spain, Iribarne-Durán et al. (2022) measured total (free plus conjugated) BP-3 in 75% of all samples with a median concentration of 0.59 ng/mL (range: 0.14 - 8.20 ng/mL). Hines et al. (2015) report milk concentrations of total (free plus conjugated) BP-3 of < LOD – 10.4 µg/L for 9 participants. In humans, the frequent detection of BP-3 (and its major metabolite BP-1) in breast milk or placental tissues implies potential transfer to fetus and breastfed infant (SCCS, 2021).

6.1.2 Animal studies

BP-3 is readily absorbed after oral, intravenous or topical skin administration to rodents (El Dareer et al., 1986; Jeon et al., 2008; Kadry et al., 1995; Okereke et al., 1993, 1994). Study summaries and further information are given in Table VIII.A2 of the Appendix.

After **oral exposure** to a single gavage dose, BP-3 is rapidly absorbed, with plasma peaks at 3–4 hrs after exposure. In the blood, BP-3 binds to proteins and the plasma elimination half-life is around 5 hrs (Jeon et al., 2008; Kadry et al., 1995). BP-3 is distributed to different organs. 6 hours after oral administration of BP-3 to male rats, BP-1 (the major metabolite) was present in most tissues, including liver, kidney, testes, intestine, spleen and skin (Okereke et al., 1993). Kadry et al. (1995) who used the same study design found the highest concentrations of total and free BP-3 in the liver.

The major metabolite of BP-3 in rodents as in humans is BP-1. Further minor metabolites are: 2,3,4-trihydroxybenzophenone (THB); 2,5-dihydroxy-4-methoxybenzophenone (2,5-DHMB) and 2,2′-dihydroxy-4-methoxybenzophenone (2,2′-DHMB or BP-8), and their corresponding glucuronide and sulfate conjugates (El Dareer et al., 1986; Kadry et al., 1995; Mutlu et al., 2020; Okereke et al., 1993).

Figure VIII.2 gives an overview on the metabolites of BP-3 identified in rodent studies.

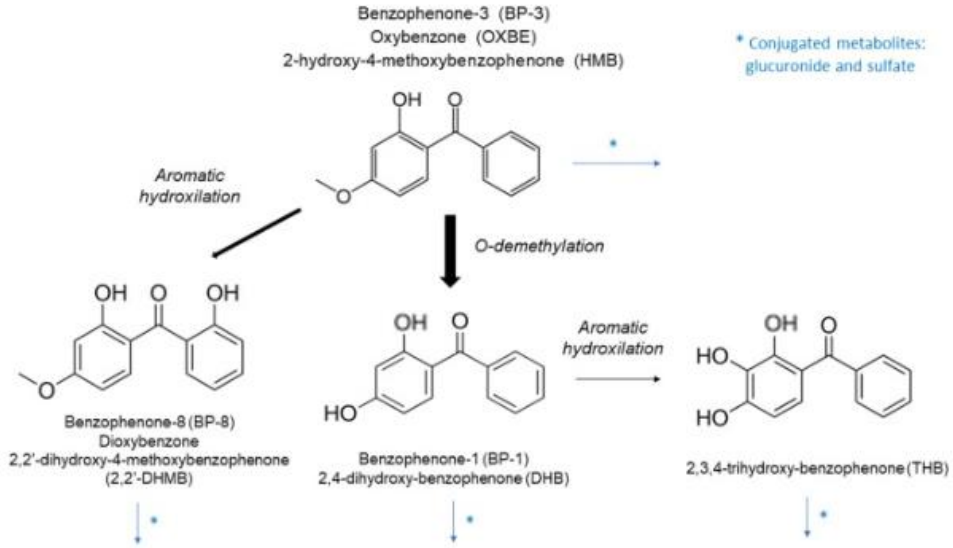


Figure VIII.2: Metabolites of BP-3 identified in rodent studies, taken from Mustieles et al. (2023)

Mustieles et al. (2023) report the rank of total analyte concentrations in plasma after high dietary exposure (10,000 and 30,000 ppm): BP-3 ≈ BP-1 > 2,5- DHMB ≫ THB. At a lower dietary exposure (3,000 ppm) the order changes to: BP-1 > 2,5-DHMB ≈ BP-3 ≫ THB (Mutlu et al., 2017). Mustieles et al. (2023) speculate that this observed difference might be due to the saturation of metabolism at higher doses, increasing the accumulation of BP-3 in plasma and tissues.

In their oral feeding study exposing female rats from gestational day (GD) 6 until postnatal day (PND) 58 to BP-3, Mutlu et al. (2017) found that all determined analyte concentrations in plasma increased with increasing dose and were significantly higher than the controls at PND 28 and PND 56. All the analytes were quantified in all plasma samples and total analyte concentrations were considerably higher than free analyte concentrations, suggesting extensive conjugation. Mean plasma concentrations of total BP-3 and BP-1 were higher (~100-300 fold) than free BP-3 and BP-1 concentrations.

Conjugation with glucuronic acid and elimination via urine is one of the major routes of BP-3 excretion (Okereke et al., 1993; Kadry et al., 1995). For example, after a single gavage administration of $^{[14C]}$BP-3 to rats it was shown that BP-3 was (with 39–57%) mainly excreted in the urine, followed by excretion via feces (24–42%) with no apparent differences between doses, or sexes (Mutlu et al., 2020).

The metabolism of BP-3 after **dermal administration** of 100 mg/kg bw in Sprague Dawley rats was investigated by Okereke et al., (1994). Absorption was rapid as the parent compound and its metabolites were detected in plasma 5 min post-administration. Peak plasma concentration of 35 ± 4.5 μg/mL (mean ± standard error of the mean, S.E.) was attained at 2.5 h post-administration. Disappearance from the plasma was biphasic with different half-lives
(1.3 h for α phase and 15.05 h for β phase). There was also extensive binding of BP-3 and its metabolites to plasma proteins. Three metabolites were identified in plasma, 2,4- dihydroxybenzophenone (DHB; BP-1) and 2,2'-dihydroxy-4-methoxybenzophenone (DHMB; BP-8) were the major metabolites detected in the plasma, while 2,3,4-trihydroxybenzophenone (THB) was detected in trace amounts. Tissue distribution studies revealed that THB was the major metabolite followed by BP-1 (both free and conjugated) in all tissues examined. The liver contained the highest amount of metabolites followed by the kidney, spleen and testes, respectively.

In a recent study by Mutlu et al. (2020) a **species and route comparison** was performed. The authors found that BP-3 was well-absorbed by rats and mice following gavage administration and moderately to well absorbed following dermal application, depending on the formulation used. BP-3 was extensively metabolized and excreted via urine and feces with minimal tissue retention. ADME properties of BP-3 were, in general, similar across rats and mice of both sexes with minor species, sex, and route differences.

Rodent-human exposure comparisons

In their review, Mustieles et al. (2023) outline rodent-human exposure comparisons. The authors report on findings by Mutlu et al. (2017) and Nakamura et al. (2015) (the latter study is described in section 6.2.4), who compared their BP-3 rodent data, using the internal concentrations, with human concentrations obtained from an intervention study conducted by Janjua et al. (2008). Nakamura et al. (2015) concluded that "exposure levels of the rats dosed with 3,000–10,000 ppm of BP-3 (equivalent to 207.1 - 670.8 mg/kg bw/day) appeared to be similar to BP-3 serum concentrations in humans". Mutlu et al. (2017) found that free BP-3 plasma concentrations in their rat toxicokinetic feeding study were within ~ 0.1–4.0-fold that of the human dermal study.

Mustieles et al. (2023) updated this rat to human ratio (RHR) by selecting the same rodent internal concentrations at PND 56 (Mutlu et al., 2017), but selecting human data from Matta et al. (2020) (see Table VIII.A1 in the Appendix). The authors selected the geometric mean Cmax value after just one whole-body lotion application (94.2 ng/mL), representing a common peak-exposure scenario (e.g., sunscreen user spending one day at the beach). Additionally, the lowest and highest BP- 3 concentrations of the C_{max} range were considered (44.6 and 177.6 ng/mL, respectively), as an approximate representation of the 10% of the population subjected to the lowest and highest dermal absorption since a high variability exists. The estimated RHRs ranged from 0.21 to 5.84 depending on the dose to which rodents were exposed through feed (3,000, 10,000 or 30,000 ppm), and the human concentration (low range, geometric mean or high range) chosen. The overall conclusion of the authors was that the calculated ratios indicate that chronic oral BP-3 doses up to 10,000 ppm (corresponding to an oral dose of ≈1,000 mg/kg bw/day) given to adult rats produce internal free BP-3 plasma concentrations that overlap with human peak

concentrations of free BP-3 in blood after sunscreen use (see Mustieles et al,. 2023 for details). Uncertainties concerning this estimation are the lack of human toxicokinetic data on internal BP-1 levels as well as differences in exposure between rats (continuous exposure) and humans (exposure through different routes and continuous low-dose level of exposure combined with intermittent peaks of exposure after use of sunscreens or PCPs, among other sources). Regarding an orientation for the interpretation of in vitro studies, the chosen human plasma free BP-3 concentrations after a whole-body application in Matta et al., (2020) [GM (range): 94.2 (44.6–177.6) ng/mL], would correspond to an in vitro exposure concentration of 0.41 (0.20–0.78) µM (Mustieles et al., 2023).

6.1.3 *In vitro* studies

In its 2008 opinion, the SCCP (SCCP, 2008) concluded, that there was 2006 no sound dermal absorption study available, allowing the calculation of the Margin of Safety for BP-3. However, the committee found a at that time newly provided *in vitro* dermal absorption study according draft OECD TG 428 (CWFG, 2007) scientifically acceptable.

BP-3 was investigated *in vitro* for its absorption and penetration properties on viable porcine skin. Therefore, two Oil-in-Water (o/w) and two Water-in-Oil (w/o) standard sunscreen formulations, containing either 2% or 6% of the UV-filter were used. 3 independent experiments were performed per formulation, each involving measurements on 6 skin samples (18 samples per formulation). Fresh dermatomed pig ear skin samples (thickness 400 ±80 µm) were treated with 10 µL/cm^2 (~ 10 mg/cm^2) of the test formulation for 24 hours under dynamic and non-occluded conditions. The stratum corneum was separated by tape stripping from the lower skin layers. BP-3 was analysed by HPLC.

After the application period of 24 hours, BP-3 was detected in the skin extracts and in the receptor solution samples. The vast majority, however, was found in the wash solution, irrespective of the investigated test item (o/w or w/o sunscreen). Since primarily during the first two experiments an impaired recovery was observed, the wash off and extraction procedure was optimized to guarantee a higher recovery in the repetition of the experiments. As 31 of the 72 samples failed to generate a BP-3 recovery of 85% at the end of the test, the results presented originate from 9 to 12 evaluable samples (each sample coming from a different donor) per formulation tested.

The authors of the study concluded that, under the experimental conditions reported, around 3-4% (between 7-18 µg/cm^2) of BP-3 (depending on the concentration in the formulation) penetrated the skin samples during 24 hours and therefore can be considered as bioavailable (SCCS, 2021). Due to some shortcomings in this study, SCCS (2021) applied an additional 2 x SD (standard deviation) to this value, yielding a **dermal absorption of 9.9%, for sunscreen products which was then used by SCCS for the calculation of the systemic exposure dose (SED)**.

For other cosmetic products, the SCCS has used a dermal absorption of 8% [mean (4%) + 2 SD (2x2%)] based on the results of the study performed with 2% BP-3.

6.2 Toxicity of benzophenone-3

6.2.1 Acute toxicity

There are no observations on humans.

The 2006 SCCP Opinion (SCCP/1069/06) stated that the studies reporting the acute toxicity values in terms of LD50-oral-rat (>6,000, 11,600 and >12,800 mg/kg bw), and LD50-dermal-rabbit (>16,000 mg/kg bw) were **not performed according to appropriate guidelines and GLP** (SCCS, 2021).

6.2.2 Irritation and sensitization

6.2.2.1 Irritation and corrosivity

Skin irritation

In its 2006 report, SCCP (SCCP/1069/06) commented that the 3 reports available at that time (ACC, 1976; CIR, 1978; Shelanski and Gittes, 1964 cited in SCCP, 2006) were very short and that limited data was provided. All 3 reports mentioned came to the conclusion that BP-3 is **not irritating to the skin**.

In a recent study, the skin irritation potential of BP-3 was investigated in an *in vitro* assay using EpiDermTM in an OECD Test Guideline 439 compliant study. The mean tissue viability after treatment with BP-3 was 97.8%. The tissue viability of the positive control (5% SDS) was 1.6%. The epidermal morphology was comparable to the human epidermis. Under the conditions of the *in vitro* study, BP-3 was determined **to be non-irritating to skin** (Han et al., 2019 in SCCS, 2021).

Mucous membrane irritation/Eye irritation

In its 2006 report, SCCP (SCCP/1069/06) commented that the 4 reports available at that time (ACC, 1976; CIR, 2003; Hazleton, 1953; Shelanski and Gittes, 1965) were very brief and that limited information was provided. All 4 reports indicated that BP-3 is **non-irritant** to the rabbit eye.

SCCP (2006) concluded that "BP-3 is not considered as being irritating to the skin and the eyes".

SCCS (2021) reconfirmed that human data with the compound under in-use conditions do not provide any indication of skin and eye irritation due to BP-3. The committee concluded that additional testing in this field does therefore not appear necessary (SCCS, 2021).

6.2.2.2 Skin sensitisation

BP-3 dissolved in Dimethylformamide (DMF) was assessed for its possible contact allergenic potential in the murine local lymph node assay (LLNA) in female CBA/CaOlaHsd mice (LLNA test, guideline test with GLP) (ECHA dissemination, 2021) (4 mice/group). In conclusion, BP-3 was shown to be not a skin sensitizer in this murine local lymph node assay. A supporting guinea pig maximisation test performed in 1991 also showed that the substance **is not skin sensitizing**.

SCCS (2021) reports on a number of studies considered in the 2008 SCCP Opinion reporting on positive photoallergic reactions to BP-3**. SCCS (2021) concludes that the results from more recent studies (and the outcome of pre-marketing human exposure tests of products containing BP-3 (NTP, 2020)) confirm the previous evaluation of SCCP that BP-3 can cause photoallergic reactions.** According to SCCS (2021) the European photo-patch test task force has stated that BP-3 is the second most frequent photo contact allergen among the UV filters (Gonçalo, 2013). In addition, **one case of anaphylactic reaction to BP-3** has been reported (Tawfik & Atwater, 2019). Study summaries and further information are given in Table VIII.A3 of the Appendix.

6.2.3 Repeated dose toxicity

6.2.3.1 Human data

For humans, no data on repeated dose toxicity of BP-3 is available.

6.2.3.2 Animal data

For animals, repeated dose toxicity data are available for the oral and dermal route for rats and mice. Study summaries and further information are presented in Table VIII.A4 of the Appendix.

The most frequently observed adverse effects following repeated oral administration of BP-3 to rats and mice were decreased total body weights, effects on the kidney (changed kidney weights, histopathologic lesions in the kidney) and liver (increase in both absolute and relative liver weights, microscopic changes associated with cytoplasmatic vacuolisation of hepatocytes) as well as effects on the reproductive system (females: estrous cycle length increased; males: epididymal sperm density decreased, abnormal sperm increased). Studies with skin administration of BP-3 to rats and mice indicated that these animals are generally similarly affected by oral and dermal exposures, but that the effects were considerably less pronounced with dermal exposure due to lower systemic exposure levels achievable by this route (French, 1992; ECHA dissemination, 2021). Toxicity to the reproductive system has been observed in rats and mice with both oral and topical administration. The most common finding in males was a decrease in epididymal sperm density (in mice as low as 23 mg/kg bw/day), and in some studies an increase in the number of abnormal sperms. In female rats and mice, a prolonged duration of the oestrus cycle was observed. These results prompted further investigation of reproductive toxicity of BP-3.

6.2.4 Reproductive toxicity

6.2.4.1 Human data

For humans, no data on reproductive toxicity of BP-3 is available.

6.2.4.2 Animal data

Nakamura et al. (2015) determined the **effects of maternal and lactational exposure to BP-3 on development and reproductive organs of offspring** of 11-13 weeks old time-mated female Harlan Sprague Dawley rats (7-8 rats/group) given 0, 1000, 3000, 10,000, 25,000, or 50,000 ppm BP-3 in feed. Average consumed doses were calculated from the amounts of feed consumed as: 0, 67.9, 207.1, 670.8, 1798.3, and 3448.2 mg/kg bw/day respectively. Exposure was from GD 6 until weaning on PND 23. No significant differences in the number of implantation sites/litter, mean resorptions/litter, % litters with resorptions, number of litters with surviving pups at PND 23, number and weights of live fetuses, or sex ratios between the control and BP-3 dose groups were observed. At PND 23, the absolute and relative kidney weights of the dams were significantly increased at 50,000 ppm and the absolute and relative liver weights were significantly increased from 10,000 ppm. Female offspring showed at PND 23 significantly decreased body and paired ovarian weights in the 25,000 and 50,000 ppm dose groups. Uterine weights were significantly decreased at 50,000 ppm.

Relative liver to body weight ratios were significantly increased and paired kidney weights were significantly decreased from 10,000 ppm. Male offspring had significantly decreased body weights at PND 23 in the 25,000 ppm and 50,000 ppm dose groups. Additionally, paired kidney weights and paired testes weights were significantly reduced at 50,000 ppm.

In the highest dose group, normalized anogenital distance in male pups was also decreased. The number of spermatocytes per seminiferous tubule was significantly reduced at doses of 3,000 ppm and higher. An overview of the determined effects can be found in Table VIII.5. Due to the decrease of spermatocytes, the SCCS considered 3,000 ppm as a LOAEL and therefore the **NOAEL is 1,000 ppm (67.9 mg /kg bw/day**). This value has been used by the SCCS (2021) as the point of departure (POD) for the MoS calculation (see section 7).

Table VIII.5: Nakamura et al. (2015), reproductive and developmental toxicity

BP-3 dose (ppm)						
	0	**1,000**	**3,000**	**10,000**	**25,000**	**50,000**
Dams						
Body weight	-	decrease in dose-dependent manner at GD 10, 15 and 20	decrease in dose-dependent manner at GD 10, 15 and 20	decrease in dose-dependent manner at GD 10, 15 and 20	decrease in dose-dependent manner at GD 10, 15 and 20, significant at GD 15	decrease in dose-dependent manner at GD 10, 15 and 20, significant at GD 10
Food consumption	-	-	-	-	significant decrease at GD 10-13 (but not at other time points)	significant decrease at GD 10-13 (but not at other time points); in postnatal period: significantly decreased at PND 14-23
Absolute and relative liver weights	-	-	-	significantly increased at PND 23	significantly increased at PND 23	significantly increased at PND 23

BP-3 dose (ppm)						
	0	**1,000**	**3,000**	**10,000**	**25,000**	**50,000**
Absolute and relative kidney weights	-	-	-	-	-	significantly increased at PND 23
Paired ovaries, uterine, and relative paired ovaries and uterine weights	-	Significantly higher (no dose-dependent effect – not seen at higher doses)	-	-	-	-
Clinical chemistry of dams						
ALT	-	-	-	-	Significantly increased at GD 10, 15, 20, and PND 23	Significantly increased at GD 10, 15, 20, and PND 23
ALP	-	-	-	elevated at all time points	elevated at all time points	elevated at all time points
Total bile acids	-	-	-	-	Elevated at GD 10, 15, and PND 23	Elevated at GD 10, 15, PND 23, and GD 20
Creatinine	-	-	-	-	Significantly decreased on PND 23	Significantly decreased on GD 15, 20, and on PND 23
Glucose, total protein, albumin and cholesterol	-	-	-	-	-	Significantly elevated at PND 23
BUN and AST	-	-	-	-	-	Significantly lower at PND 23
Reproductive/pregnancy parameters						
Number of implantations sites/litter	-	-	-	-	-	-
Mean resorptions/litter and % litters with resorptions at GD 10, 15, and 20	-	-	-	-	-	-
Number of litters with surviving pups at PND 23	-	-	-	-	-	-
Number or weights of live fetuses	-	-	-	-	-	-
Sex ratios at GD 20	-	-	-	-	-	-
Malformations	-	-	-	-	-	1 fetus had hydrocephaly
Male offspring (PND 23)						

BP-3 dose (ppm)						
	0	**1,000**	**3,000**	**10,000**	**25,000**	**50,000**
Body weight gain	-	-	-	-	-	Decreased by PND 14
Body weight	-	decreased in dose-dependent manner	decreased in dose-dependent manner	decreased in dose-dependent manner	Significantly less than control	Significantly less than control
Normalized anogenital distance (AGD)	-	-	-	--		Significant reduction
Paired testes weight	-	decreased in dose-dependent manner	decreased in dose-dependent manner	decreased in dose-dependent manner	decreased in dose-dependent manner	decreased in dose-dependent manner, significantly decreased
Testes/body weight ratio	-	-	-	-	-	-
Paired epididymides weights	-	-	-	-	Lower but not statistically significant	Lower but not statistically significant
Absolute and relative liver weights	-	-	-	-	-	-
Paired kidney weights	-	-	-	-	-	Significantly decreased
Relative paired kidney weights relative to body weight ratio	-	-	-	-	-	-
Nipple retention	-	-	-	-	-	-
Testes descent	-	-	-	-	-	-
Clinical chemistry at PND 23						
Serum ALT	-	-	-	-	Decreased	Decreased
Cholesterol levels	-	-	-	-	Significantly increased	Significantly increased
Testis morphology at PND 23						
Number of spermatocytes per seminiferous tubule	-	-	Significantly reduced	Significantly reduced	Significantly reduced	Significantly reduced
Number of Sertoli cells per seminiferous tubule	-	-	-	-	-	-
Apoptotic cells in testis	-	-	Many apoptotic cells	Many apoptotic cells	Many apoptotic cells	Many apoptotic cells
Effects on serum testosterone levels at PND 23						

BP-3 dose (ppm)						
	0	**1,000**	**3,000**	**10,000**	**25,000**	**50,000**
Serum testosterone levels	-	-	Significantly lower (according to study authors not treatment related)	-	Significantly lower (according to study authors not treatment related)	-
Female offspring at PND 23						
Body weight gain	-	-	-	-	-	Decreased by PND 14
Body weight	-	-	-	-	Significantly less than control	Significantly less than control
Paired ovarian weights	-	-	-	-	Significantly decreased	Significantly decreased
Ovarian/body weight ratio	-	-	-	-	-	-
Uterine weight	-	-	-	-	-	Significantly lower
Uterine/body weight ratio	-	-	-	-	-	-
Relative liver to body weight ratio	-	-	-	Significantly increased	Significantly increased	Significantly increased
Paired kidney weight				Significantly lower	Significantly lower	Significantly lower
Paired kidney weight to body weight ratio	-	-	-	-	-	-
Normalized AGD	-	--	-	-	-	-
Effects on ovarian histology at PND 23						
Number of small, medium, or large follicles	-	-	-	-	--	
Number of antral follicles	-	-	-	Significantly lower	-	Significantly lower
Clinical chemistry at PND 23						
Serum ALT	-	-	-	-	Decreased	-
Cholesterol levels	-	-	-	-	Significantly increased	Significantly increased

Modified One-Generation study

In a modified one-generation (MOG) study of the National Toxicology Program (NTP), Sprague Dawley SD rats were exposed to BP-3 in feed at 3,000 ppm, 10,000 ppm, and 30,000 ppm. Exposure of F0 females to BP-3 began on GD 6 (implantation). F1 offspring were exposed to the same exposure concentration as their respective dams. The NOEL for adult general toxicity is 3,000 ppm based on increases in kidney weights and histopathological findings in the urinary system consistent with chronic obstructive nephropathy.

There is equivocal evidence of reproductive toxicity of BP-3 based on a decrease in F2 litter size and there is some evidence of developmental toxicity of BP-3 based on observed postnatal growth retardation. The no-observed-effect level (NOEL) for BP-3-related effects on body weight is 3,000 ppm based on lower body weights in both sexes in both generations. According to the authors of the study, exposure to BP-3 was not associated with effects consistent with alterations in estrogenic, androgenic, or antiandrogenic action.

The aforementioned effect on body weight contributed to the apparent BP-3-related decreases in male reproductive organ weights (NTP, 2022). Partial results of the study appear to have already been available to SCCS 2021. An Overview of relevant oral reproductive toxicity studies with BP-3 is given in Table VIII.A5 of the Appendix.

6.2.5 Mutagenicity / Genotoxicity

In its 2021 opinion, the SCCS refers to the SCCP opinions of 2006 and 2008 (SCCP/1069/06, SCCP/1201/08), which pointed out that: '*As far as the (photo)mutagenic/(photo)genotoxic potential of BP-3 was concerned, the presented in vitro and in vivo assays indicated that the substance did not possess (photo)mutagenic or (photo) genotoxic properties*.' NTP (2020) states that bacterial mutagenicity tests conducted in *Salmonella typhimurium* strains TA98 and TA100 as well as in *Escherichia coli* strain WP2 uvrA pKM101, with and without rat liver S9, were negative.

In SCCS (2021) a new Mammalian Cell Gene Mutation Test in Chinese hamster lung fibroblasts is described. BP-3 **did not induce mutations at the HPRT locus of V79 cells** in the presence or absence of S9-mix (ECHA dissemination, 2021). This suggests that BP-3 is not likely to be mutagenic under the conditions tested.

Majhi et al. (2020) studied the effects of BP-3 on DNA damage in human breast epithelial cells. They used immunostaining with antibodies against gamma-H2AX and 53BP1. Formation of R-loops was determined with DNA:RNA hybrid–specific S9.6 antibody. The authors found that concentrations of 1 µM or 5 µM BP-3 increased DNA damage similar to that of E2 treatment in an ERα-dependent manner. BP-3 exposure caused R-loop formation in a normal epithelial cell line when ERα was introduced. R-loops and DNA damage were also detected in mammary epithelial cells of mice treated with BP-3.

However, the SCCS (2021) noted that the biological significance of these findings was not clear. Although proof of the formation of R-loops and DNA double strand breaks was provided after exposure to BP-3, it is not certain how this translates into a mutagenic hazard of BP-3. The overall SCCS conclusion was that the newly provided data on gene mutations at the HPRT locus in V79 cells also **support the safety of BP-3 in terms of genotoxicity**.

Study summaries and further information are presented in Table VIII.A6 of the Appendix.

6.2.6 Carcinogenicity

NTP (2020) report on two-year studies in both mice and rats. The animals were exposed to BP-3 concentrations between 0 and 10,000 ppm in feed. NTP (2020) concluded that *"under the conditions of these 2-year studies, there was equivocal evidence of carcinogenic activity of BP-3 exposure in male Hsd:Sprague Dawley® SD® rats based on the occurrence of brain and spinal cord malignant meningiomas. There was equivocal evidence of carcinogenic activity in female Hsd:Sprague Dawley® SD® rats based on the increased incidence of thyroid C-cell adenomas and the increased incidence of uterine stromal polyps* (see also Table VIII.A7 in the Appendix). *There was no evidence of carcinogenic activity in male or female B6C3F1/N mice at exposure concentrations of 1,000, 3,000, and 10,000 ppm. Increases in the incidences of non-neoplastic lesions of the testis and pancreas in male rats and of the uterus and adrenal cortex in female rats occurred with exposure to BP-3. Increases in the incidences of non-neoplastic lesions of the bone marrow (males and females), spleen (males and females), kidney (males and females), and liver (males) in mice occurred with exposure to BP-3."* For details see Table VIII.A8 in the Appendix.

In its 2021 opinion, the SCCS agreed with the NTP that BP-3 has '**equivocal evidence of carcinogenic activity'**. This level of evidence is the third level within five categories and is defined as "a marginal increase of neoplasms that may be chemical related" (SCCS, 2021).

6.2.7 Photo-induced toxicity

Phototoxicity/photoirritation and photosensitisation

In its 2021 report SCCS references SCCP (2006): "*Benzophenone-3 has been extensively tested for its **photoirritating** potential in vitro during the validation of the 3T3 Neural Red Uptake Phototoxicity (NRU PT) test and **was found negative in the majority of cases**. (…)*

In addition, the submission contains a number of reports of clinical trials with regard to the photoallergenic potential of UV-filters in general. In each of these, a number of clear positive reactions to Benzophenone-3 are described. In the current report, some extra references on this issue have been added by the SCCP to the ones included in the submission. Looking at the positive photoallergic reactions to Benzophenone-3, it must be emphasized that the study population in all tests consisted of patients with a suggested history of photocontact allergy. As a general rule, results of clinical trials should be followed up in order to detect potential trends towards an increasing incidence of (photo)allergic reactions to specific compounds.

*In the case of BP-3, the presented publications clearly indicate that the UV-filter **is a photoallergen**.*"

In SCCS (2021) an *in vitro* 3T3 NRU PT test is described (Spielmann et al., 1998). The authors concluded that BP-3 was not phototoxic in the 3T3 NRU assay in the presence and absence of sunlight.

SCCS (2021) comments that the results indicate that BP-3 is **not phototoxic**.

Photomutagenicity / photoclastogenicity

In SCCP (2006) an *in vitro* **bacterial mutation assay** (study year: 2004) is described (Chemie Wirtschaftsfördergungs GmbH, unpublished data). The overall conclusion of the authors was that BP-3 did not induce gene mutations by base pair changes or frameshifts in the genome of the bacterial strains used and was therefore shown to be **non-photomutagenic** in this *Salmonella typhimurium* photomutagenicity test.

***In vitro* photomutagenicity: chromosome aberration test**

Taken from SCCP 2006 "As far as the (photo)mutagenic/(photo)genotoxic potential of Benzophenone-3 is concerned, the presented in vitro and in vivo assays indicate that the substance does not possess (photo)mutagenic or (photo) genotoxic properties." No more recent data have been made available to SCCS, therefore SCCS 2021 considers that this conclusion is still valid. Further information is given in Table VIII.A9 of the Appendix.

6.2.8 Neurotoxicity

6.2.8.1 Human data

There are no study reports included in the registration dossier (ECHA dissemination, 2021).

In their review, Mustieles et al. (2023) report four analysable **epidemiological studies** investigating associations between BP-3 exposure and neurodevelopmental endpoints (see Appendix Table VIII.A14). No associations were reported between prenatal BP-3 exposure and cognitive function in two studies. While one study reported no association with behaviour, another study found that prenatal BP-3 exposure was associated with an increased risk of prosocial problems[46] in the study group (Guo et al., 2020).

6.2.8.2 Animal data

Mustieles et al. (2023) report in their review on an *in vivo* study which has investigated the effects of BP-3 exposure on neuronal development (Pomierny et al., 2019). The authors remark however, that clear signs of dose response and of adversity are missing. In a developmental study in SD rats with gestational and pubertal exposure (100 mg/kg bw applied dermally), BP-3 raised oxidative stress and induced apoptosis in the brain. BP-3 increased the concentration of extracellular glutamate in examined brain structures and changed the expression of glutamate transporters. In analyses of behavioural effects, it was observed that BP-3 had no effect on short-term memory but impaired offspring's spatial memory.

[46] High levels of empathy and prosocial behaviours are sometimes associated with anxiety and depression. From early on in development extreme concern may be a sign of these internalizing problems.

6.2.8.3 *In vitro* studies

Some *in vitro* studies found evidence confirming the possibility of BP-3's neurotoxic activity (reviewed in Mustieles et al., 2023). Study summaries are given in Table VIII.A10 of the Appendix.

6.2.9 Endocrine-related adverse effects

Based on potential endocrine disruption, BP-3 was included in the Community Rolling Action Plan (CoRAP) list by ECHA (ECHA 2014). In its 2021 opinion SCCS notes that:

- BP-3 has not been identified under REACH regulation as a Substance of Very High Concern (SVHC) for its ED properties neither for the environment nor for human health.

- Danish Centre on Endocrine Disrupter Evaluation: Suspected ED in Category 2a (Hass et al., 2012). SCCS/1625/20 Final Opinion on Benzophenone-3 (CAS No 131-57-7, EC No 205-031-5)

- In February 2019, FDA considered BP-3 in category III: not safe for use as UV filter. FDA considered that inadequate data on BP-3's developmental and reproductive toxicity were reported. The literature suggests that BP-3 may have endocrine activity (see, e.g., Schlumpf et al., 2001; Schlumpf et al., 2004; Krause et al., 2012).

6.2.10 Endocrine Mode of Action

The endocrine mode of action is described in detail in SCCS (2021) and Mustieles et al. (2023) and summarized here briefly. Further information is presented in Table VIII.A11 of the Appendix.

In silico modelling

Two studies using in silico modelling yielded contradictory results. Hong et al. (2016) used consensus classification modelling that classified substances into ER binders and non-binders based on two Decision Forest (DF) models built using two different training datasets. The DF models did not take into account possible metabolic activation/deactivation of the compounds. BP-3 was predicted as ER non-binder. In contrast, Sosnovacvá et al. (2016) reported in silico assessment using QSAR Toolbox to predict for the compounds investigated for potential to bind on ER. In this study BP-3 was predicted to have a strong binding affinity for the ER binding site.

In vitro

Estrogenic activity

Weak to moderate estrogenic activity of BP-3 through both Estrogen Receptor Alpha (ERα) and Estrogen Receptor Beta (ERβ) has been observed in various cell-based reporter gene assays (reviewed in Mustieles et al., 2023). In studies that additionally tested BP-1, BP-1 was found to be between 4 and 100 times more potent than BP-3 (Kunz and Fent, 2006; Miller et al., 2001; Molina-Molina et al., 2008; Suzuki et al., 2005; Watanabe et al., 2005). Heneweer et al. (2005) reported an increase in estrogen-regulated pS2 gene transcription in MCF-7 cells at low concentrations, both for BP-3 and BP-1, presenting EC_{50} values (half-maximal effective concentrations) of 0.5 and 0.12 µM.

Anti-androgenic activity

Antagonism of the androgen receptor (AR) has been shown in several cell-based reporter gene assays, both at moderate and high concentrations (reviewed in Mustieles et al., 2023). The potency of BP-1 to exhibit AR antagonism is in most cases higher (Kunz and Fent, 2006; Molina-Molina et al., 2008; Suzuki et al., 2005).

Thyroid hormone disruption

Schmutzler et al. (2007) identified BP-3 as thyroid receptor agonist. Slight effects were observed at 1 µM (Hofmann et al., 2009), which is supported by an effect on expression of thyroid hormone regulated genes at 10 µM in rat pituitary (GH3) cells (Lee et al., 2018).

Other endocrine effects

Rehfeld et al. (2016, 2018) reported that BP-3 directly affects the motiliy of human sperm cells in vitro, showing an EC_{50} of 4.7 µM. Mustieles et al. (2023) speculate that this effect is likely linked to a mechanism involving binding to

and displacement of progesterone from a membrane bound progesterone receptor (CatSper)[47] and agrees with the BP-3-induced repression of progesterone receptor transcription found by Scheurs et al. (2005).

In vitro endpoints related to female reproduction were adversely affected by BP-3. Mouse oocyte maturation and development to the blastocyst stage was inhibited by BP-3 at 0.5 µM (Jin et al., 2020); further, BP-3 disrupted rat follicular cell population and oocyte number at the lowest effect concentration of 6 nM (Santamaria et al., 2019); further, BP-3 altered milk protein synthesis and transcriptional regulation during mammary gland differentiation at 1 pM (Altamarino et al., 2020).

In a rat uterine cytosolic ER competitive binding assay, Blair et al. (2000) determined BP-3 to be an ER non-binder (half-maximal inhibitory concentration (IC50) >1.00 x 10-4 M).

BP-3 did not show any induction of proliferation at concentrations of the order of micromolar in MCF-7 breast cancer cells and estrogen-responsive CHO cells (Watanabe et al., 2015; Kerdivel et al., 2013 reported in SCCS, 2021). At least three of the metabolites of BP-3 exhibite estrogenic potencies: BP-1, BP-8 and THB (Nakagawa, 2002; Jeon et al., 2008).

The effects of BP-1 and other UV-filters on migration and invastion of human breast cancer cell lines *in vitro* was investigated by Almamar and Darbre (2018). BP-3 showed oestrogenic activity in gene expression assays, but was not able to increase MCF-7 proliferation.

In vivo

The results from uterotrophic assays in rodents are inconsistent. Whereas Schlumpf et al., (2001) reported weak activity for BP-3 with immature Long-Evans rats, no uterotrophic response was observed in ovariectomized (surgical removal of one of both ovaries) rats when tested up to 1 g/kg (NTP, 2020).

In fish (fathead minnows), BP-3 did not show any induction potential of vitellogenin (Kunz et al., 2006).

The effects of BP-3 on the expression pattern of the estrogen receptors ERα and ERβ, the estrogen-related receptor-1 (ERR1), and the aryl hydrocarbon receptor (AhR) were investigated by Schlecht et al. (2004) in adult ovariectomized rats. After a five-day treatment with the chemicals BP-2 and BP-3 in two different dosages with a 17β-estradiol and a negative control group, the expression of the four receptors ERα, ERβ, ERR1 and AhR mRNA was measured in the pituitary, uterus and thyroid. Additionally, some "classical" markers of estrogenic action were tested. While a significant increase in uterine weight was observed in both treatment groups with BP-2, no effect was seen in the BP-3 treatment groups. BP-3 inhibited ERα transcript expression and also mildly the ERβ gene expression in the uterus. Whether these effects are of functional significance for the uterus remains unknown at present and deserves further investigation. SCCS (2021) summarizes "*In brief, unlike BP-2, an estrogen-like effect was not observed for BP-3, although regulatory effects on receptors expression were observed indicating potential endocrine disruption that is not assessed by "classical" estrogen markers."*

Majhi et al. (2020) investigated the estrogenic effects of BP-3 *in vitro* in different cell lines, and in ovariectomized BALB/c mice (7-9 weeks old) administered orally with BP-3 for four days at a dose of 3 mg/kg bw/day. The results (taken from SCCS, 2021) are reported here:

The results showed that in T47D cells, treatment with BP-3 at 1 µM or 5 µM increased γ-H2AX intensity in comparison with the control (p <0.0001), but no dose-dependent increase was observed (1µM vs 5 µM BP-3). In MCF-7 cells, a dose-dependent increase in nuclear γ-H2AX intensity was observed after treatment with BP-3 in the range of 1–30 µM. BP-3 treatment (1–5 µM) showed increased nuclear 53BP1 intensity over control in both T47D and MCF-7, but only MCF-7 showed dose-dependent increase. The effect on γ-H2AX was however contrasted with the mRNA expression of estrogen-responsive gene TFF1, as responses to 5 µM BP-3 did not differ significantly from the control. In comparison, treatment with 10 nM E2 stimulated a 13.1-fold increase in the expression of TFF1. Based on the measures of transcriptional activity in MCF7 human breast cancer cell lines, the authors concluded that typical exposures to BP-3 appear to pose a minimal risk for breast cancer through ER-mediated transcriptional activation of target genes.

[47] CatSper is a transmembrane Ca^{2+} channel for which progesterone is the endogenous ligand to open the channel – not to be confused with traditional progesterone receptor.

Other endocrine MoA

Disrupting effects of BP-3 towards progesterone (PR) were reported by Schreurs et al. (2004). Although BP-3 did not activate PR, it is the antagonist of PR as revealed by PR CALUX1 bioassay. As evaluated by an *in vitro* reporter system containing a duplicated thyroid hormone response element of the HLA-DR4 serotype, BP-3 can induce luciferase activity, showing agonistic activity towards Thyroid Hormone Receptor (THR) (Schmutzler et al., 2007).

BP-3 was evaluated in quantitative (dose-response) high throughput screening assays by NTP in the Toxicology in the 21st Century (Tox21) program, and significant activity was observed in assays measuring stimulation of ER, progesterone receptor, constitutive androstane receptor, pregnane X receptor, retinoic acid receptor, and estrogen-related receptor signaling pathways (NTP report, 2020).

6.2.11 Endocrine effects in humans

Janjua et al. (2007) investigated the effect of BP-3 and two other UV filters on the hypothalamic-, pituitary-thyroid axis in humans after whole body topical application. In this 2-week single-blinded study 15 young men (age range 23-29 years) and 17 postmenopausal women (age range 54-86 years) were assigned to daily whole-body topical application (2 mg/cm^2) of a basic cream in week 1 and with a mixture of the three sunscreens at 10% (w/w) each in week 2. For each male study participant, the daily amount of cream applied over 4 days was 40 ±3 g, for each female participant the amount was 35 ±3 g. All study participants were healthy and did not take any regular medication. A blood test performed 4 weeks before enrolment verified thyroid and postmenopausal status. Hormone levels were determined by commercially automated immunoassay systems. No biologically significant effects on hormone levels (TGB, TSH, T4 total, T4 free, T3 total, T3 free) were observed, indicating that the concentrations of sunscreen compounds absorbed were not capable of disturbing the homeostasis of thyroid hormones in adult humans.

Epidemiological studies

The results of epidemiological studies investigating the relationship between maternal prenatal BP-3 exposure and **birth weight** are inconsistent. Eight mother-child cohorts were identified by Mustieles et al. (2023) (Wolff et al., 2008; Philippat et al., 2012; Philippat et al., 2019; Tang et al., 2013; Ferguson et al., 2018; Krause et al., 2018; Messerlian et al., 2018; Aker et al., 2019) which studied the relationship between maternal prenatal urinary BP-3 concentrations and birth outcomes that found a possible sex-specific association towards higher birth weights (BW) in boys, and lower BW in girls. This was particularly the case when exposure was measured in the third trimester. Other studies found null associations with BW or an inverse association with gestational age.

Eight epidemiological studies were found that investigated **reproductive hormones, fertility and female related reproductive endpoints** (Buck Louis et al., 2014; Buck Louis et al., 2018; Minguez-Alarcón et al., 2019; Arya et al., 2020; Pollack et al., 2018; Scinicariello and Buser, 2016; Adomnei et al., 2018; Joensen et al, 2018;). Pollack et al. (2018) reported associations of BP-1 and BP-3 with increased progesterone and with increased ES, FSH, and LH serum levels. Mustieles et al. (2023) comment that if these findings are confirmed in subsequent studies, they might have implications for fertility and hormonally-mediated chronic diseases. Arya et al. (2020) reported on the results of a cross-sectional study from the NHANES survey in 789 menopausal women. It was found that the cluster represented by BP-3, triclosan and bisphenol A was associated with a history of infertility – but no individual compound showed this association alone. In two longitudinal preconception cohorts (Buck Louis et al., 2014; Buck Louis et al., 2018), a possible association between couple's BP-3/BP-1 exposure and fecundity could not be supported.

Concerning **female diseases**, BP-3 exposure was also reported to be associated with increased endometriosis risk (Kunisue et al., 2012; Peinado et al., 2020), however, these associations were more evident with BP-1. Further, a non-significant association was reported between higher urinary BP-3 and BP-1 concentrations and a suggestive higher risk of uterine fibroids (or Leiomyomas – non-cancerous tumors growing in the muscle tissue of the uterus) (Pollack et al., 2015). Results from two case-control studies after diagnosis reported null associtations with the risk of polycystic ovary syndrome (Gu et al., 2019) and with breast cancer (Parada et al., 2019).

Investigations on **male reproductive health** were performed in five studies/cohorts. In cross-sectional data from the NHANES survey higher urinary BP-3 concentrations were associated with lower serum total testosterone (TT) levels in male adolescents (Scinicariello and Buser, 2016). Another study reported positive associations between

urinary BP-1 and BP-3 concentrations and serum FSH levels. BP-1 concentrations were associated positively with the TT/E2 ratio and negatively with the inhibin B/FSH ratio (Adomnei et al., 2018). In a study with participants who carried the Filaggrin gene (FLG) loss-of-function mutation and a control group without this mutation, higher urinary BP-3 concentrations were associated with higher TT and E2 serum levels, these associations were not found in the control group. (Joensen et al., 2018). No associations with semen quality parameters were found in the preconception LIFE cohort when BP-1 and BP-3 concentrations were determined in urine or seminal plasma (Buck Louis et al., 2015; Smarr et al., 2018). Non-significant inverse associations were reported between paternal preconception seminal plasma BP-1 and BP-3 concenctrations and fecundity (Buck Louis et al., 2018). In a study from China in males with idiopathic infertility no associations between urinary BP-3 concentrations and sperm quality were found (Chen et al., 2013).

Child anthropometry and puberty

Mustieles et al. (2023) report on six studies which analysed anthropometric outcomes and puberty in girls. Main findings from these studies are: third trimester urinary BP-3 concentrations were inversely associated with body fat mass in girls, but not in boys (4-9 years old) from North America (Buckley et al., 2016). In a study with 1017 girls from the US (Deierlein et al., 2017), baseline BP-3 exposure was inversely associated with waist circumference (but not BMI). This was observed from the age of 10 onwards, statistical significance was reached at 12 and 13 years. Further, girls in the fifth quintile of BP-3 concentrations showed a significantly delayed breast development (Wolff et al., 2015) which is consistent with the lower adiposity among girls in response to BP-3 exposure. Another study (Binder et al., 2018) found that urinary BP-3 concentrations at the age of 8 – but not 11 – were associated with an earlier age at menarche amond Chilean girls. Whereas non-significant trends between higher prenatal and peripubertal urinary BP-3 concentration and an earlier age at menarche was reported in another study (Harley et al., 2019). In 440 pubertal girls from NHANES no cross-sectional associations with age at menarche were found (Buttke et al., 2012).

In boys, baseline urinary BP-3 concentrations were reported to be inversely and longitudinally associated with stages of testicular volume (Huang et al., 2020) which is consistent with the inverse association between BP-3 and serum testosterone reported for NHANES adolescent males.

Other possible mechanisms may comprise alterations in DNA methylation. In a small pilot study from Denmark (Almstrup et al., 2020) with Danish pubertal children, an interesting marker was identified. The thyroid hormone receptor interactor 6 gene (TRIP6) is mainly induced in testicular Leydig cells during puberty. In the study from Denmark, BP-3 exposure was associated with higher TRIP6 DNA methylation, and lower TRIP6 serum protein levels.

Thyroid hormone biomarker

Mustieles et al. (2023) report on seven studies describing associations between exposure to BP-3 and thyroid hormone levels. In the LIFECODES cohort from the USA (Aker et al., 2018), pregnant women provided urine and blood samples at four times during pregnancy. Urinary BP-3 was inversely associated with total triiodo-thyronine (TT3) levels at all visits except the second one, and with free thyroxine (FT4) levels at all visits except the fourth one. Further, urinary BP-3 concentrations tended to be positively associated with thyroid stimulating hormone (TSH), especially at the second and third visits. Another study with pregnant women from Mexico (Berger et al., 2018) found that average prenatal urinary BP-3 concentrations were inversely associated with serum FT4 and total thyroxine (TT4) levels in women, and with lower TSH in neonates.

In pregnant women from Puerto Rico (Aker et al., 2016), an inverse association between the perinatal average urinary BP-3 concentrations and serum FT3 levels was reported. In adult participants from the NHANES study (Kim et al., 2017), urinary BP-3 concentrations were cross-sectionally and inversely associated with decreased TT3 and FT3 levels, but higher TSH serum levels. In the same study population, BP-3 concentrations were inversely associated with TT4 in males, while a non-significant but positive association was observed with TT3 serum levels in females.

Mustieles et al. (2023) conclude, that overall, the associations between urinary BP-3 concentrations and thyroid hormone levels found in human studies support an inhibitory effect of BP-3 and/or BP-1 on thyroid hormone synthesis.

6.2.12 Endocrine effects in animals

Recently a few *in vivo* studies have investigated ED-related effects of BP-3, these include: Nakamura et al., 2015 (see 6.2.4 Reproductive toxicity), Laplante et al., 2018; Matouskova et al., 2020; Majhi et al., 2020 and Santamaria et al., 2020). These studies are reported in SCCS (2021) and main findings are included here, comments by SCCS (2021) are also presented. A study by Kariagina et al. (2020) is included in Mustieles et al. (2023) but not in SCCS (2021), and is also briefly summarized here.

Study summaries and further information are given in Table VIII.A12 of the Appendix.

The study by **Nakamura et al. (2015)** has already been described in section 6.2.4. The authors determined the effects of maternal and lactational exposure to BP-3 on developmental and reproductive organs of offspring of time-mated female Harlan Sprague-Dawley rats (11- to 13-week-old; 7-8/group) dosed with 0, 1000, 3000, 10,000, 25,000, or 50,000 ppm BP-3 added to chow. Average consumed doses were calculated from the amounts of feed consumed as: 0, 67.9, 207.1, 670.8, 1798.3, and 3448.2 mg/kg/day respectively. Exposure was from GD 6 until weaning on PND 23. Findings from Nakamura et al. (2015) are summarized in Table VIII.5. The number of spermatocytes per seminiferous tubule was significantly reduced at doses of 3,000 ppm and higher. Due to this effect, the SCCS considered 3,000 ppm as a LOAEL and therefore the **NOAEL is 1,000 ppm (67.9 mg /kg bw/day).**

The studies by LaPlante et al. (2018) and Matouskova et al. (2020) presented in the following are both from the same laboratory and have used the same species and doses.

Laplante et al. (2018) examined the long-term effects of exposure to BP-3 on the morphology of the mammary gland in female six- to 8-week-old ovariectomized **Balb/C mice** exposed during pregnancy and lactation. The study consisted of an acute exposure part and a chronic part. In the acute exposure study, BP-3 did not alter the weight of the uterus (unlike 17b-estradiol), but produced modest but substantial effects on expression of ERa (Esr1) in the uterus, consistent with an estrogenic response. In the chronic exposure study BP-3 did not show effects on the number of pubs, and induced only modest (non-statistically significant) changes to the length of gestion. In mice exposed to 3000 µg/kg/day a substantial reduction in ductal density of the mammary gland was observed. The two highest doses of BP-3 similarly induced an intermediate phenotype for expression of progesterone receptor. A monotonic, dose-dependent increase in cell proliferation was also observed in the BP-3-treated females, becoming statistically significant at the highest dose.

According to LaPlante et al. (2018), these data suggest that BP-3, at doses relevant to human exposures, produces long-lasting alterations to mammary gland morphology and function. However, further studies are needed to determine if exposure to this chemical during pregnancy and lactation will interfere with the known protection that pregnancy provides against breast cancer. The authors conclude that the results presented are consistent with prior studies indicating that **BP-3 is estrogenic but with lower potency than other known estrogenic endocrine disruptors**.

Matouskova et al. (2020) investigated the endocrine-disrupting potential of BP-3 on male and female 6-8 – week BALB/c mice. The authors evaluated the effects of oral exposures to BP-3 on the growth and morphology of the mammary gland, body weight and anogenital distance in BALB/c mice exposed to 30, 212 or 3000 µg/kg/day *in utero* and during lactation.

Exposure to BP-3 during the developmental phase reduced the size and growth of mammary gland in males prior to and during puberty. In exposed females, BP-3 reduced mammary cell proliferation, decreased the number of cells expressing estrogen receptor α, and altered mammary gland morphology in adulthood. The authors conclude that their results suggest exposure to BP-3 during the developmental phase **alters growth parameters in the mouse mammary gland, findings consistent with what would be expected if BP-3 was a hormonally active endocrine disruptor**.

SCCS (2021) commented on these two studies and concluded that whilst the studies **may indicate potential endocrine activity of BP-3, they do not provide any clear scientific evidence that may be used in safety assessment of BP-3**. SCCS (2021) listed some shortcomings, e.g., LaPlante et al.; (2018) did not report the content of phytoestrogens in the diet used in the study and whether it could have influenced the results. Other effects reported in the chronic study at lower doses (212 µg/kg/day or 30 µg/kg/day) are either statistically non-

significant or a result of modification in the expression of certain genes. It is also not clear whether or not the observed effects on the mammary gland were reversible or whether these could lead to some adversity.

Further, SCCS (2021) has noted that the results presented in these studies are often in contradiction with the authors' conclusions in regard to dose response, non-monotonic dose response, estrogen and progesterone time of response etc, and do not demonstrate a clear correlation between the treatment and the measured parameters.

Majhi et al. (2020) carried out an *in vivo* study in ovariectomised BALB/c mice, administered orally with BP-3 (3,000 µg/kg/d) and 17β-estradiol (250 µg/kg/d). The nuclear c-H2AX intensity in the mammary gland of both BP-3 and 17β-estradiol treated animals was noted to be significantly higher than of animals treated with vehicle control. However, whilst 17β-estradiol led to stimulated proliferation of mammary epithelium and increased uterine weight, no significant effects were observed for BP-3.

Santamaria et al., (2020) investigated how BP-3 affects the progeny of mice after dermal exposure during early pregnancy. Mice (female C57BL/6J and male BALB/c) were **dermally** exposed to BP-3 at the dose of 50 mg/kg bw/day during early gestation. Critical parameters of pregnancy such as implantation rate, intrauterine embryonic and fetal development were investigated. BP-3 (but not its metabolites) was also measured in blood samples at GD6 and in blood and amniotic fluid in GD14 during the first pregnancy and at GD10 and GD14 during the second pregnancy.

After the first pregnancy, no differences between BP-3 treated and control mice on the number of implantations, abortions, and abortion rates were reported, placental parameters were not different between groups. Fetal weight in BP-3 treated mice was significantly reduced at GD14 compared to control mice and placental weight was not modified, therefore the feto-placental index was reduced. 16.13% of foetuses in the BP-3 group were under the 5th percentile meaning that they are affected by intrauterine growth restriction. Hemodynamic parameters of the maternal uterine artery showed altered pattern at GD10 but not at other time point, male offspring from exposed mice were lighter at PND4 and then from PND10 onwards. On the other hand, females born to BP-3 exposed mice showed decreased weight at PND1, PND10 and PND13, but then recovered their normal weight from PND16 forward. Thus, decreased weight persisted until weaning only in males. The sex ratio was altered with a higher proportion of females than males in the offspring of BP-3 treated mice (84% versus 64% in control group).

After the second pregnancy, no effects were reported on implantations and abortion numbers. The placenta weights from BP-3 treated mice were significantly reduced at GD14 but not at GD10. Fetal weight and fetus/placenta index showed no difference between controls and BP-3 treated mice. No modification of the weight curves of the offspring were reported. The sex ratio was altered with more females than males in the offspring of BP-3 treated mice.

The authors concluded that the results suggest dermal exposure to BP-3 at a low internal dose during early pregnancy could impair fetal development, resulting in growth-restricted fetuses. The intrauterine growth restriction (IUGR) phenotype of the fetuses was associated with transient changes in hemodynamic parameters at maternal uterine artery, reduced weight of male offspring and persistence of significant levels of BP-3 in amniotic fluid until GD14. Further, the authors remarked that there was an alteration of the sex ratio of the progeny, with females predominating over males when analyzing both pregnancies.

SCCS (2021) commented on the study by Santamaria et al. (2020).

- A decrease in fetal weight and feto-placental index was observed on GD14 during 1st pregnancy though placental weights were not affected. No significant effects were observed during 2nd pregnancy. The magnitude of these effects was limited.
- Any significant differences between BP-3 treated and control groups in terms of blood velocity parameters of the maternal arteria uterina (UA) were observed only on GD-10 and not on other days (GD5, GD8, GD12 and GD14). These differences included end diastolic velocity (in control mice), and systolic/diastolic ratio, resistance index and pulsatility index in treated mice.
- A decrease in the weight of offspring in the BP-3-treated group was noted during 1st pregnancy - from the mid-lactation until weaning in both male and female pups, and after birth. The magnitude of these effects was limited.

- Change in sex ratio of the offspring was observed in BP-3-treated group, with a higher proportion of females in both the 1st and 2nd pregnancies of mothers (that were exposed to BP-3 only during GD0 to GD6 of the first pregnancy), compared to the vehicle-treated controls. The percentage of males and females from both pregnancies was combined into one figure. After reviewing the individual data provided by the authors upon SCCS request, the SCCS concluded that this finding has limited statistical significance because the number of litters is too low to make any robust conclusion. Only few other publications have investigated alterations in sex ratio following the exposure of test animals to chemicals.

In conclusion, the SCCS considers that whilst this study indicates a potential reproductive effect of BP-in mice, the **observations need to be consolidated through further research**, and for the time being it does not provide any clear scientific evidence that can be used in the safety assessment of BP-3.

Kariagina et al. (2020) (not reported in SCCS (2021) investigated the effects of BP-3 in combination with a diet high in saturated animal fat (HFD) on mammary tumorigenesis in BALB/c mice, using the *Trp53-null* transplantation modell. The authors found that in combination with a high fat diet, 70 mg BP-3/kg bw/day increased mammary tumor cell proliferation and decreased tumor cell apoptosis. This stated dose elicits levels of BP-3 excretion in urine similar to that observed in humans with heavy topical application of BP-3-containing sunscreen. The authors conclude that future studies are needed to identify the mechanistic basis for BP-3 effects on mammary tumorigenesis and how dietary fat interacts with BP-3 to alter outcomes.

Overall conclusions on the ED properties of BP-3

In their review, Mustieles et al. (2023) concluded that there is substantial *in vitro* evidence to identify BP-3 as a chemical with endocrine disruption potential, and moderate evidence from *in vivo* studies, in which endocrine-related outcomes were found at low and moderate doses (French, 1992; Kariagina et al., 2020; LaPlante et al, 2018; Matouska et al., 2020; Santamaria et al., 2020).

SCCS (2021) however, states on a largely identical data basis that the currently available evidence for endocrine disrupting properties of BP-3 **is not conclusive, and is at best equivocal**. This applies to the data derived from *in silico* modelling, *in vitro* tests and *in vivo* studies, whether considered individually or collectively. There are either contradictory results from different studies, or the reported data do not show dose-response relationship, and/or the effects are seen only at relatively very high doses that can only be considered far beyond the human exposure range. In view of this, the SCCS considers that whilst some studies suggest that BP-3 may have endocrine effects, the evidence is not conclusive enough at present to derive a new endocrine-related toxicological point of departure for use in safety assessment.

For the calculation of the margin of safety (MoS), the study from Nakamura et al. (2015) was selected by SCCS (2021). **Due to the effects on the spermatocytes, which may be due to ED effects of BP-3, the SCCS considered 3,000 ppm as a LOAEL and 1,000 ppm as the NOAEL (67.9 mg/kg bw/day)**.

6.2.13 Combined effect with other substances

The relationship between BP-3, octocrylene and ketoprofen is outlined in de Groot and Roberts et al. (2014). Ketoprofen is a non-steroid anti-inflammatory drug (NSAID). In several countries commercial topical products contain 2.5-5% ketoprofen. De Groot and Roberts (2014) report in their review that in a considerable number of patients with a history of reactions to ketoprofen and/or who have a positive photopatch test reaction to ketoprofen, concomitant photo-reactions to octocrylene and – to a lesser degree - BP-3 have been observed. Many patients who are photoallergic to octocrylene **co-react to BP-3**, a number of the cases may be attributable to ketoprofen photosensitization, and can be explained by photo-cross reactivity to this NSAID.

Kunz and Fent (2006) assessed in a recombinant yeast assay carrying the human estrogen receptor alpha (hERalpha) mixtures of two, four and eight UV filters alone, or in combination with 17 β estradiol (E2), at different effect levels and no-observed-effect-concentrations (NOEC). Predictions of the joint effects of these mixtures were calculated by employing the concentration addition (CA) and independent action (IA) model. Most binary mixtures comprising of pure hERα agonists showed a **synergistic activity at all mixture combinations**. Only in combination with benzophenone-1, antagonistic activity was observed at some effect levels. All mixtures of four or eight, pure or pure and partial hERα agonists, alone or including E2, showed synergistic activity at concentrations giving an increase of 10% of basal activity (BC10). This occurred even at concentrations that were at the NOEC level of each single compound. Hence, there were substantial mixture effects even though each UV filter was present at its NOEC level. These results show that significant interactions occur in UV filter mixtures, which is important for the hazard and risk assessments of these personal care products.

7 Evaluation of BP-3 by various bodies and conclusion

DNEL derivation based on registration dossier

Derived no effect level (DNEL) values as provided in the registration dossier (ECHA Dissemination, 2021) are summarized in Tables VIII.6 and VIII.7. BP-3 was investigated by NTP (National Toxicology Program, US, French et al., 1992; NTP, 1992) for subchronic toxicity towards rats and mice by using oral and dermal exposure (see section 6.2.3.2). Systemic effects were seen in rats and a NOAEL oral of 393 mg/kg bw/day (females) and 429 mg/kg bw/day (males) respectively was derived. The NOAEL found with mice was more than a magnitude higher (7579 mg/kg bw/day females and 5981 mg/kg bw/day males) but also showed liver and kidneys being the target organ. The DNEL was set to 2 mg/kg bw/day for the general population. Also, the dermal route was investigated for both species and the DNEL was set to 20 mg/kg bw/day for the general population as systemic availability by dermal route was limited. The DNEL for inhalation was set to 6.8 mg/m^3 for the general population.

Table VIII.6: DNELs for the general population for BP-3 (ECHA dissemination, 2021)

Route of exposure	DNEL general population	Study basis
Long term exposure		
inhalation	6.8 mg/m^3	Overall assessment factor (AF[48]): 50; NOAEC: 342 mg/m^3 using bioavailability of 100% for the oral and 100% for the inhalation route and assuming that rat oral and inhalation absorptions are equal to human oral and inhalation absorption, a NOAEC corr of 393 mg/kg bw/day / 1.15 m^3/kg = 342 mg/m^3 is used.
dermal	20 mg/kg bw/day	NOAEL: 3 930 mg/kg bw/day; overall AF: 200; using bioavailability of 100% for the oral and 10% for the dermal route and assuming that rat oral and dermal absorptions are equal to human oral and dermal absorption.
oral	2 mg/kg bw/day	NOAEL: 393 mg/kg bw/day; overall AF: 200;

The DNELs for workers for long-term exposure are presented in Table VIII.7. The data base used for the POD is the same as for the general population.

[48] AF = assessment factor (assessment factors are used to address the differences between the experimental data and the human situation, taking into account the uncertainties and variability in the extrapolation procedure and in the available data set)

Table VIII.7: DNELs workers for BP-3 (ECHA dissemination, 2021)

Route and duration of exposure	DNEL workers	Study basis
Long term exposure		
Inhalation	27.7 mg/m^3	Overall assessment factor (AF): 25 NOAEC: 693 mg/m^3; using bioavailability of 100% for the oral and 100% for the inhalation route and assuming that rat oral and inhalation absorptions are equal to human oral and inhalation absorption, a NOAEC corr of 393 mg/kg/day / 0.38 m^3/kg * 6.7/10 = 693 mg/m^3 is used.
Dermal	39 mg/kg bw/day	Route of original study: oral; overall AF 100; NOAEL: 3 930 mg/kg bw/day; [using bioavailability of 100% for the oral and 10% for the dermal route and assuming that rat oral and dermal absorptions are equal to human oral and dermal absorption, thus the corrected dermal NOAEL is 3930 mg/kg bw/day]

POD used by the SCCS

SCCS (2021) used the NOAEL of 67 mg/kg bw/day as POD for the determination of a MOS (Margin of Safety). This POD from the reproductive toxicity study by Nakamura et al. (2015) is based on a reduction of the number of spermatocytes per seminiferous tubule in offspring at doses of 207.1 mg/kg bw/day and higher.

There had been a focus on endocrine disrupting effects in the 2021 SCCS opinion. However, due to several uncertainties/shortcomings in these studies e.g., contradictory statements, SCCS (2021) concluded that although there is evidence from some *in vivo* studies to suggest that BP-3 may have endocrine effects, this is not conclusive enough to establish a POD related to endocrine-disrupting properties as a starting point for safety assessment.

8 Selected biomarkers and analytical method for BP-3

Although BP-3 can be biotransformed to several metabolites, exposure to BP-3 can be assessed by measuring the total (free plus conjugated) concentrations of BP-3 in urine (Calafat et al., 2008). Thus, a non-invasive determination of BP-3 exposure in humans is possible.

On-line TurboFlow-LC-MS/MS method has been developed at Copenhagen University Hospital for simultaneous biomonitoring of nine UV filters in urine (benzophenone, benzophenone-1 benzophenone-2, benzophenone-3, 5-chloro-2- hydroxybenzophenone, 4-hydroxybenzophenone, 4-methyl-benzophenone, 3-(4- methylbenzylidene)-camphor, and 3-benzylidene camphor) (Frederiksen et al., 2017).

9 Derivation of HBM-GVs for BP-3

The database for benzophenone-3 is not sufficient to derive an HBM-GV based on the relationship between human internal concentration of the exposure biomarker(s) and related health effects. Furthermore, no toxicological reference value proposed by a European or relevant non-European institution (e.g. TDI, RfD) suitable for the derivation of HBM-GVs was found.

Therefore, the derivation of HBM guidance values is based on findings from toxicity studies on animals and additionally on human toxicokinetic studies. Critical effects that are considered relevant for the derivation of HBM-GVs are in particular the reduction of the number of spermatocytes per seminiferous tubule in offspring, but also the reduction in body weight (gain), observed in studies on reproductive and developmental toxicity. **Since there is no data giving the indication for a rodent-specific mechanism, it can be assumed that these effects are also relevant for humans.**

9.1 Choice of key study, POD and Assessment Factors

The great majority of *in vivo* toxicity studies was performed using oral exposure. However, a few studies used subcutaneous/dermal exposure. Results of the 13 weeks oral and dermal studies indicate that rats and mice are generally **similarly affected by oral and dermal exposures** (ECHA dissemination, 2021).

We follow SCCS (2021) and regard the study by Nakamura et al. (2015) as key study. In this reproductive and developmental toxicity study in female rats a **NOAEL of 67.9 mg/kg bw/day** for PND 23 male offspring was derived based on a reduction of the number of spermatocytes per seminiferous tubule in offspring at doses of 207.2 mg/kg bw/day and higher (Nakamura et al., 2015).

The **derivation of HBM guidance values for BP-3 or its metabolites from an oral study is justifiable** for the following reasons.

1. due to the areas of application of BP-3, it can be assumed that exposure peaks are the result of large-scale dermal application, but the background exposure of the population may also be oral (hand-mouth contacts, lipstick application) and possibly also inhalation (use of sprays containing BP-3). Mustieles et al. (2023) conclude that peak-exposures are through the use of sunscreen and cosmetics, while a continuous low-dose exposure is linked to food, textiles, indoor air and dust.

2. derivation of HBM guidance values based on animal studies with dermal administration is considered uncertain because of differences in dermal absorption in rodents and humans.

The animal POD was then extrapolated to a human equivalent (external) dose (Toxicological Reference Value - TRV) with the application of a standard overall assessment factor of 100 to account for inter- and intraspecies variabilities, hereby following the ECHA guidance chapter R.8 (European Chemicals Agency) (ECHA, 2012).

9.2 Calculation of HBM-GVs for the general population for BP-3

For the derivation of the HBM guidance value, the urinary mass balance approach was applied (Apel, 2020). This method can be applied to substances that are primarily eliminated through urinary excretion, and for which regular repeated exposure is likely. For such substances, the approach assumes a balance between the substance intake and the substance excretion, reaching a steady-state in the urine matrix. Under this assumption, the urinary excretion rate is a constant fraction of the intake rate (Angerer et al., 2011).

The HBM guidance values for the general population were calculated from the TRV, using Equation 1:

Equation 1: HBM − GV (GenPop) = TRV ∗ Fue (substance) / daily urine excretion rate adjusted to bw

with parameters defined as follows:

Fue: substance-specific steady-state fraction of urinary excretion

According to Woutersen et al. (2022) and Rousselle et al. (2022) the Fue was obtained from two human experimental exposure studies (Hayden et al., 1997; Sarveiya et al., 2004) (see section 6.1.1). Hayden et al. (1997)

report a BP-3 output in urine of 1-2% of the initial dose, 10 hours after initial dermal application to nine healthy adult volunteers. Sarveiya et al. (2005) report up to 1% BP-3 of the initial dose dermally applied measured in urine after 48 hours. Woutersen et al. (2022) decided to incorporate a Fue of 0.01 (1%) as the more conservative value. As there is no reliable dermal toxicity study available nor a conversion factor from oral to dermal, it had to be assumed that absorption and metabolism are the same in both routes.

Daily urine excretion rate adjusted to body weight in L/kg bw/day

The daily urinary flow rate which is currently used as standard is 0.035 L/kg bw/d for women and 0.030 L/kg bw/d for children and men (German HBM Commission, 2025, submitted).

Table VIII.9 presents an overview of the information and parameters incorporated in the calculation of the HBM-GV_{GenPop}.

Table VIII.9: Overview of input variables for the derivation of HBM-GV_{GenPop} for BP-3

Parameter	Value	Source
Animal PoD	NOAEL = 67.9 mg/kg bw/day	Nakamura et al., 2015 (also used by SCCS, 2021)
Overall AF	AF = 100	ECHA R.8 guidance
Fue	1% or 0.01	Hayden et al. (1997); Sarveiya et al. (2004)
Urine volume per kg bw and day	0.030 L/kg bw/day for children and men; 0.035 L/kg bw/day for women	German HBM Commission (2025, submitted)

Thus applies: HBM-GV_{GenPop} for children and men = (0.679*0.01)/0.03 = 0.226 mg/L (rounded)= 226 µg/L

HBM-GV_{GenPop} for women = (0.679*0.01)/0.035 = 0.194 mg/L = 194 µg/L

The HBM-GVs are preferably given in relation to volume and not in relation to creatinine, as the physiological creatinine formation in healthy adults varies according to gender and is significantly reduced with age. Creatinine formation in children is also significantly lower and, moreover, strongly age-dependent. Meat consumption also contributes as an external factor to the creatinine pool. These aspects should be considered when adjusting biomarker measurements in urine for creatinine (Carrieri et al., 2001; Commission Human Biomonitoring of the German Environment Agency, 2005; Sallsten and Barregard, 2021; Abraham et al., 2023).

Note on the provisional HBM-GV derived under HBM4EU

Woutersen et al., 2022 used the same POD and Fue but incorporated the HBM4EU default average daily creatinine excretion in the derivation of the creatinine corrected provisional HBM guidance value. The HBM4EU default creatinine value is based on the calculation in (Aylward et al., 2009) and amounts to an average creatinine excretion of 1.4 g/L per day for both sexes combined. Adjusted to the default bodyweight of 70 kg, as defined by ECHA R.8 and followed by HBM4EU, this amounted to an average creatinine excretion rate of 0.02 g/kg bw/day. With these defaults a provisional HBM-GV of 0.34 mg/g creatine = 340 µg/g creatinine was derived.

9.3 Level of confidence attributed to the derived HBM-GVs

As mentioned in the HBM4EU strategy paper to derive HBM-GVs (Apel et al., 2020), it is suggested to attribute a level of confidence (low, medium or high) to each calculated HBM-GV. The level of confidence (LoC) should reflect the uncertainties identified during the elaboration of the value and could constitute a good incentive to revise later on the value(s). Attributing low levels of confidence for the criteria described below may help to highlight the data gaps and to address them to the scientific research community. However, a low level of confidence does not necessarily mean a low level of protection, because the HBM-GV derivation is based on very conservative scenarios and default assumptions (Apel et al., 2020).

It should be noted that the LoCs proposed in this document are not determined according to an established recognised methodology, but rather rely on expert judgment regarding the reliability of the data and the calculation method used to derive the HBM-GV values.

The overall LoC attributed to the HBM-GV_{GenPop} for BP-3 is set to "**medium**'.

The following considerations are taken in extracts from HBM4EU (2022a) Annex to the deliverable D5.11 "human biomonitoring in risk assessment: 4th set of examples on the use of HBM in risk assessments of HBM4EU priority chemicals" (Woutersen et al., 2022):

The POD is derived from an oral study in rats, while the Fue is based on dermal absorption and excretion in humans. Also, the most important route of exposure to BP-3 in real life is likely to be dermal. As there is no reliable dermal toxicity study suitable for the derivation of a point of departure, nor an appropriate study that compares both routes, it is assumed that the absorption and metabolism are the same in both routes.

The urinary mass balance approach assumes a balance between substance intake and substance excretion and, accordingly, derives the HBM guidance value by application of the Fue as a steady-state metabolic conversion factor (Angerer et al., 2011). In reality, substance excretion may vary based on intake-rate and half-life of elimination. The limited toxicokinetic information from both animal and human studies does not point to rapid elimination from the body, which creates uncertainty in the Fue value.

Rat studies show biphasic elimination with alpha half-life 0.88 – 1.3 hours and beta half-life 15.05 – 15.90 hours (Kadry et al., 1995; Okereke et al., 1994). Matta et al. (2019) report a long terminal half-life in humans with a mean range of 24-31 hours for BP-3. The detection of BP-3 in human breast milk and adipose tissue might suggest possible build-up of BP-3 in humans (Schlecht et al., 2004; Wang et al., 2015).

More substance-specific toxicokinetic data is required to improve the reliability of the Fue including data on blood levels over time. Compared to several other chemical substances, the current Fue seemed to be on the low side (Asimakopoulos et al., 2014). Asimakopulous et al. (2014) incorporated a Fue of 2%, based on Hayden et al. (1997), in the transformation of internal estimates to external intake estimates. As the current study transformed external intake estimates to internal exposure levels, a Fue of 0.01 (1%) was considered the more conservative value. In case the Fue was in reality higher, the current HBM guidance value is slightly overestimating the risk ratio.

10 Factsheet HBM-GV_{GenPop} for BP-3

Table VIII.10: Data sheet for proposed derivation of HBM-GVs for BP-3

Parameter	Value / Descriptor	Comments
HBM-GV_{GenPop}	HBM-GV_{GenPop} for children and men = 226 µg/L urine women = 194 µg/L urine	Biomarker of exposure: total BP-3 in urine
Level of confidence	Medium	See note for explanation
Status	Agreed	
Year of issue	2025	
EC-No.	205-031-5	
CAS No	131-57-7	
Classification by registrants	H400: Very toxic to aquatic life. H411: Toxic to aquatic life with long lasting effects.	
Molar mass	228.26 g/mol	
Identification exposure biomarkers	Total (conjugated and free) BP-3	Total (conjugated and free) BP-3 concentration is commonly measured in urine as a non-invasive biomarker for internal exposure
Molar mass of biomarker(s)	228.26 g/mol	
Key studies for TK data, authors, year	Hayden, 1997; nine healthy humans (mean age 29 years) received one single dose of in practice 13·0 (1·00) g of sunscreen product The formulation remained unoccluded for 12 h before being removed with soap and water. It is estimated that the actual amount absorbed from the applied formulation over a 10-h period was between 1 and 2% of the applied amount contained in the product.	
Type of study	Human male and female volunteer study (n=9)	
Route of exposure	Dermal administration	
Half-life of biomarker(s)		
Fraction urinary excreted (Fue(10h))	1-2%	
Derivation method	**Based on animal POD**	
Key study, Author(s), Year	Nakamura et al., 2015	
Species	Female Harlan Sprague-Dawley rats and their offspring	
Route/type of study	Oral, reproductive toxicity	
Exposure duration	Chronic	

Parameter	Value / Descriptor	Comments
Critical endpoint	Reproductive toxicity (reduction of the number of spermatocytes per seminiferous tubule in offspring after exposure of the mothers at higher doses (i.e., doses of 207.1 mg/kg bw/day and higher)	
POD Value	NOAEL = 67.9 mg/kg bw/day	Nakamura et al., 2015, (taken also by SCCS in its opinion (2021))
assessment factors	: (10 x 10)	Intra- and interspecies differences ECHA R.8 guidance
TRV (- like) value	0.679 mg/kg bw/day	
Calculated HBM-GV_{GenPop}	Children and men = 226 µg/L urine Women = 194 µg/L urine	mass balance approach, based on urinary volume

Note:

- Level of confidence of the HBM-GV_{GenPop}: reflects the reliability of the proposed HBM-GV. The level of confidence takes into account the various uncertainties underlying the derivation of the HBM-GV (e.g. reliability of the key study used to derive the TRV/TRV-like value, uncertainties related to the extrapolations leading to the TRV/TRV-like value, to toxicokinetic data on the substance of interest, and to the calculation of the final HBM-GV). Attribution of a global level of confidence for the proposed benzophenone-3 HBM-GV_{GenPop} is suggested, considering:
- the nature and quality of the benzophenone-3 toxicological data: benzophenone-3 (2-hydroxy-4-methoxy-benzophenone; BP-3) is extensively studied for regulatory purposes as well as out of scientific interest. No data on repeated dose toxicity on BP-3 are available for humans. Therefore, the assessment of toxicity is based only on animal studies. Several reproductive and developmental toxicity studies on rats via the oral route of exposure have been conducted. As there is no conversion factor from oral to dermal available, it had to be assumed that absorption and metabolism are the same in both routes. The tocikokinetic data used for the HBM-GV calculation is based on one human volunteer study on only 9 adults, thus data on children are lacking.→ **low/medium confidence**
- the critical endpoint and mode of action: the evidence of the BP-3 effects on reproduction/development is based on some animal studies via the oral route of exposure, this is adequate, since in addition to dermal exposure, human hand-mouth and lipstick exposure can also be assumed. There is no data giving the indication for a rodent-specific mechanism, thus it can be assumed that these effects are also relevant for humans. However, no evidence for the specific effects is available from epidemiological studies. → **low/medium confidence**
- the selected key study: reproductive toxicity study in rats after oral administration of BP-3 (Nakamura et al., 2018)→ **medium/high confidence**
- the selected POD: NOAEL is available from original study→ **high confidence**
- the extrapolations leading to the TRV-like value: AFs for inter-species and intra-species variability → **medium confidence**
- Altogether the global level of confidence for the BP-3 HBM-GVs in the general population is set to '**medium confidence'**.

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12 Appendix to Deliverable AD 4.3 - part VIII

Derivation of human biomonitoring guidance values for Benzophenone-3

Table VIII.A1: Overview toxicokinetics and metabolism in humans

Reference	Test performance, results, evaluation
Gonzalez et al., 2006	• Percutaneous absorption of BP-3 after repeated whole-body sunscreen applications was investigated, with and without ultraviolet irradiation. • A commercially available sunscreen containing 4% BP-3 was applied topically for five days by 25 volunteers. Each volunteer received 2 mg/cm² of sunscreen according to his or her BSA. The amount of sunscreen per application varied among the participants from 26 g to 47 g. The total amount of BP-3 varied between 10.4 g and 18.8 g. • There was a large variation in the total amount of BP-3 excreted in the urine. Even after compensating for the differences in BSA and thus the amounts applied, the variation was substantial. In relative terms, the total amount of urinary excreted BP-3 during the 10-day period ranged from 1.2 to 8.7% (mean 3.7%) of the total amount applied (the nonconjugated part of BP-3 was between 0.1% and 0.7%). UV exposure did not significantly affect the urinary excretion of BP-3. • In its 2006 opinion SCCP (2006) concludes that since BP-3 was combined with two other UV-filters and it was not tested at the maximum requested concentration of 10% and other routes of excretion have not been considered, no conclusion can be drawn with regard to the dermal absorption of BP-3. In its 2008 opinion **SCCS (2008) concluded** that the study contained **too many shortcomings** to generate scientifically acceptable results for the current risk assessment of BP-3 (among others: the composition of BP-3 containing sunscreens is not indicated, no individual or summary values were supplied, the application was performed by the volunteers and not under controlled conditions, there was no control measure on urine samples). Study limitations: • BP-3 metabolites (including BP-1 and BP-8) were not measured. • BP-3 was only measured in urine.
Gustavsson et al., 2002	• 11 volunteers (22-37 years), single application with sunscreen containing 4% BP-3, the average total amount excreted during 48-h was 11 mg, median 9.8 mg, which is approximately 0.4% of the applied amount; some of the volunteers still excreted BP-3 48h after the application Study limitations: • Small sample size; internal plasmatic levels not evaluated.
Hayden et al., 1997	- Nine healthy human volunteers applied a commercially available sunscreen product to the entire surface of their forearms. - In practice 13.0 (1.00) g of sunscreen product was applied to a surface area of 1051 (60.8) cm2. The formulation remained unoccluded for 12 h before being removed with soap and water. The sunscreen product was a lotion containing BP-3 6% (w/v), octyl methoxycinnamate 7.5% (w/v), octyl salicylate 5% (w/v) and octocrylene 7% (w/v), all being chemical sunscreening agents. The volunteers collected urine just before application and for 48 h after application, recording the time and volume of each sample. **Significant amounts of BP-3 penetrated the epidermal barrier**. Analysis of the urine using glucuronidase suggested that BP-3 and its metabolites (2,3,4-trihydroxy-benzophenone and 2,4-dihydroxybenzophenone) undergo extensive conjugation in the body. It was estimated that **the actual amount absorbed from the applied formulation over a 10-h period was between 1 and 2% of the applied amount contained in the product**.

Reference	Test performance, results, evaluation
	Study limitations: • Small sample size; chemical method with low sensitivity; quantitative data for each blood and urine sample was not presented in the manuscript
Janjua et al., 2008 (2007,2004)	• 32 volunteers (15 young males and 17 postmenopausal females); 4 days treatment, daily whole-body application of 2 mg/cm^2 of sunscreen formulation at 10%(w/w); • BP-3 blood concentrations and first morning urine concentrations were determined. • Maximum median BP-3 plasma concentrations were 187 ng/mL reached at 4 h in females and 238 ng/mL reached after 3 h in males. • Median plasma BP-3 concentrations at 24 h and 96 h after the first application of the active formulation were not significantly different. • In females (but not in males), median urinary BP-3 concentration significantly increased from 24h to 48h and then remained constant. • In females, urine maximum median concentrations of 44 ng/mL BP-3 were found, in males 81 ng/mL BP-3. Study limitations: • The absorption of three UV filters was tested at the same time, • the AUC was not calculated, BP-3 metabolites (including BP-1) were not assessed in urine.
Matta et al., 2019	- Randomized clinical trial with18 healthy participants; - Topical application of 3 sunscreen products (i.e. spray 1 (n = 6 participants), spray 2 (n = 6), lotion (n = 6)); The concentrations of BP-3 were 6% in spray 1, 5% in spray 2 and 4% in the lotion. Two milligrams of sunscreen per cm^2 were applied to 75% of the body surface area 4 times per day for 4 days, and 30 blood samples were collected over 7 days from each participant. - All 3 products with BP-3 resulted in internal exposure to BP-3, with plasma concentrations exceeding 20 ng/mL on day 7. Geometric mean maximum plasma concentrations were 209.6 ng/mL (coefficient of variation, 66.8%) for spray 1, 194.9 ng/mL (coefficient of variation, 52.4%) for spray 2, and 169.3 ng/mL (coefficient of variation, 44.5%) for lotion. Plasma concentrations exceeded 0.5 ng/mL within 2 hours after a single application on day 1. The mean range of terminal half-life observed was 24-31 hours. Study limitations: • The study was conducted with multiple applications of sunscreen products, urinary BP-3 and BP-1 concentrations were not evaluated, percentage of absorption had not been determined.
Matta et al., 2020	• Investigation of systemic absorption and pharmacokinetics of BP-3 (and 5 other UV-filters) in sunscreen products under single- and maximal-use conditions by repeated dermal application. The UV-filters were formulated as a lotion (4% weight/weight) or spray (6% weight/weight) (12 healthy participants/group). • Sunscreen products were applied at 2 mg/cm^2 to 75% of body surface area at 0 hours on day 1 and 4 times on day 2 through day 4 at 2-hour intervals, and blood samples were collected over 21 days from each participant. Skin samples were collected by tape stripping on days 7 and 14. • BP-3 concentrations were 258.1 (range: 131.3-498.1) ng/mL for the lotion and 180.1 (range: 70.1-476.9) ng/mL for the aerosol spray. • The maximum plasma concentrations of BP-3 after single application (day 1) were 94.2 (range: 44.6-177.6) ng/mL and 85.4 (range: 34.8-401.2) ng/mL for lotion and aerosol spray, respectively.

Reference	Test performance, results, evaluation
	• On day 7 the geometric mean levels of BP-3 in skin were 358.5 (range: 40.7-5815.2) ng/cm^2 and 698.5 (range: 114.2-6734.8) ng/cm^2, on day 14 the geometric mean levels were 18.2 (range: 3.9-341.3) ng/cm^2 and 29.1 (range: 7.0-979.6) ng/cm^2 for lotion and aerosol spray, respectively. • Cmax was achieved at day 4 (258.1, range: 131.3-498.1 ng/mL for the lotion and 180.1, range: 70.1-476.9 ng/mL for the spray). From day 5 plasma concentrations started to decrease, but were still detectable in some participants at day 21 (1.3, range: 0-14.4 ng/mL for the lotion and 2.0, range: 0.6-8.0 ng/mL for the spray). The terminal half-life was 75.8 (range: 41.8-135.3) hours for the lotion and 79.2 (range: 27.4-206.7) hours for the spray. • Following tape-stripping, BP-3 was detectable in skin with greater amounts detectable on day 7 than on day 14. The authors conclude that the continued presence of BP-3 in skin at days 7 and 14, the long terminal half-life typically exceeding 48 hours, and remaining detectable through day 21 suggest **absorption through skin is the rate-limiting step** Study limitations: • The study was conducted with multiple applications of sunscreen products; • Urinary BP-3 and Bp-1 concentrations were not evaluated.
Sarveiya et al., 2004	• 3 female volunteers; • Investigation of the absorption and distribution of sunscreens following topical application, among them BP-3. A commercially available sunscreen product was applied at a dose of 2 mg/cm^2 to the arms and back of three female human volunteers, aged 22–42 years. This constituted an application of approximately 1.7 g of the sunscreen formulation applied to a total area of approximately 860 cm^2. The lotion contained 6% BP-3 and 3 other UV-filters as active ingredients. Baseline blood and urine samples were collected prior to sunscreen application. Permeation of sunscreen into the skin, systemic absorption and urinary elimination were monitored for up to 48 h following application. At 30 min after application skin strip samples were taken. Blood samples were taken from all subjects at pre-application baseline and at 1, 2, 4, 6, 8, and 24 h post-application. Urine output of all subjects over the 48 h post-application period was collected. • Systemic absorption of BP-3 was confirmed through detection of BP-3 in the blood/plasma and in the urine. The plasma was only measured for the four sunscreens, excluding their metabolites. Up to approximately **1% of the applied dose of BP-3 were detected in urine as BP-3 and its metabolites**. The major metabolite was 2,4-dihydroxybenzophenone (DHB; BP-1), whereas 2,3,4-trihydroxybenzophenone (THB) was detected only in trace amounts. Study limitations: • Only very small sampe size, chemical method with low sensitivity, quantitative data for each blood and urine sample collected was not presented.

Table VIII.A2: Overview toxicokinetics and metabolism in animals

Reference	Findings
El Dareer et al., 1986	• Fischer 344 rats were dosed orally, intravenously or topically • BP-3 was well absorbed and excreted primarily in the urine, for either oral or intravenous doses, rats rapidly excreted the radioactivity from BP-3, with 88-105% of the dose appearing in urine and feces in 72 h. • Im summary, for rats dosed with BP-3, there was an extensive absorption of the compound followed by rapid metabolism and elimination. Only trace amounts of radioactivity derived from BP-3 persisted in the tissues.
Jeon et al., 2008	• Male Sprague–Dawley rats were dosed orally (100 mg/kg body weight, bw), dissolved in corn oil. • BP-3 was converted into at least three intermediates, including 2,4-dihydroxybenzophenone (DHB), which was formed via o-demethylation and subsequently converted into 2,3,4-trihydroxybenzophenone (THB), and 2,2-dihydroxy-4-methoxybenzophenone (DHMB), which was formed via the aromatic hydroxylation of BP-3 (i.e., type B UV filters). After a single oral dose of BP-3, the C_{max} of BP-3 reached 21.21±11.61μg/mL within 3 h (Tmax), and then declined rapidly. The concentration of the metabolites in rat blood decreased much more slowly over time compared to the parent compounds.
Kadry et al., 1995	• Male Sprague–Dawley rats were dosed orally (100 mg/kg body weight, bw), dissolved in corn oil. • Absorption from the gastronintestinal tract was rapid because BP-3 was detected in blood 5 min after administration. The peak plasma concentration (C_{max}) was 25.6 ± 4.6 μg/mL and the time of occurrence (t_{max}) was 3.0 ± 0.4 h. The half-life of absorption of BP-3 was 0.71 h. The elimination pattern was biphasic with alpha and beta half-lives of elimination of 0.88 and 15.90 h, respectively. The results of this study indicate the presence of strong binding between plasma protein and BP-3. • Tissue distribution studies at 6 h indicate that the liver contained the highest concentration of free (58.9 ± 23.8 μg) and total (free + bound or conjugated) BP-3 (2087 ± 60.1 μg), followed by kidney and testes, respectively. • Urine and feces analysis indicate that urine was the major route of excretion, followed by feces. Further • analysis of urine samples also indicates that conjugation of BP-3 with glucuronic acid was essential for the systemic elimination of the compound.
Okereke et al., 1993, 1994	• **Okereke 1993:** male Sprague-Dawley rats were orally administered BP-3 (100 mg/kg bw). • 2,4-Dihydroxybenzophenone (DHB), 2,2'-dihydroxy-4-methoxybenzophenone (DHMB), and 2,3,4-trihydroxy-benzophenone (THB) metabolites were identified as free and conjugated forms by HPLC analysis. BP-3 was rapidly absorbed, metabolized, and detected in plasma (free and protein bound) at 5 min. post administration. The parent compound and metabolites (free and conjugated) were detected at 6 hr in most tissues. DHB was present in most tissues with the highest concentration in the liver. DHMB was only detected as the conjugated form in liver, spleen, and heart. Trace amounts of THB were also detected in biological samples. Urine was the primary route, whereas feces was the secondary route of elimination of BP-3 and its metabolites. This study revealed O-dealkylation as the major pathway of BP-3 metabolism. • **Okereke 1994**: dermal administration of 100 mg/kg bw to Sprague-Dawley rats • Blood samples were collected at various intervals and the parent compound and its metabolites were analyzed by HPLC.

Reference	Findings
	• Absorption was rapid as the parent compound and its metabolites were detected in plasma 5 min post-administration. Peak plasma concentration of 35 ± 4.5 µg/mL (mean ± standard error of the mean, S.E.) was attained at 2.5 h post-administration. Disappearance from the plasma was biphasic with different half-lives (1.3 for α phase and 15.05 h for β phase). There was also extensive binding of BP-3 and its metabolites to plasma proteins. Three metabolites were identified in plasma, 2,4-dihydroxybenzophenone (DHB) and 2,2'-dihydroxy-4-methoxybenzophenone (DHMB) were the major metabolites detected in the plasma, while 2,3,4-trihydroxybenzophenone (THB) was detected in trace amounts. Tissue distribution studies revealed that THB was the major metabolite followed by DHB (both free and conjugated) in all tissues examined. The liver contained the highest amount followed by the kidney, spleen and testes, respectively.
Mutlu et al., 2017	• A liquid chromatography–tandem mass spectrometry method was developed and evaluated to quantitate free (unconjugated) BP-3 and DHB, and total (combined conjugated and unconjugated) BP-3, DHB, THB and 2,5-DHMB. The method was successfully applied to quantitate these analytes in plasma from postnatal day 28 and 56 male and female Harlan Sprague Dawley rat pups following perinatal dietary exposure to 0 (control), 3,000, 10,000 and 30,000 ppm BP-3 beginning on gestational Day 6. • All determined analyte concentrations increased with increasing dose and were significantly higher than the controls at both timepoints. All the total analytes were quantified in all plasma samples and total concentrations were considerably higher than free, suggesting extensive conjugation. • Mean concentrations of total BP-3 and DHB were higher (~100–300-fold) than the free BP-3 and DHB concentrations, and total concentrations in plasma were approximately BP-3≈DHB > 2,5-DHMB ≫ THB. • Free and total analyte plasma concentrations were not sex-dependent and in general, both free and total analytes were detected in the control samples.
Mutlu et al., 2020	• Distribution and metabolism of $^{[14C]}$BP-3 in rats and mice was assessed following **single gavage** administration (10, 100, or 500 mg/kg), single **iv administration** (10 mg/kg), or **dermal** application (0.1, 1, 10, or 15 mg/kg). • Following gavage administration, $^{[14C]}$BP-3 was well absorbed and excreted mainly in urine (39–57%) and also in feces (24–42%) with no apparent difference between doses, species or sexes. BP-3 was distributed to the tissues, however it was not retained in tissues. • Distribution of BP-3 following dermal application was comparable to that following gavage administration; no differences between doses, species, or sexes were observed but absorption varied depending upon vehicle. Light paraffin oil had the highest absorption and excretion (98% of the BP-3 dose absorbed). • Urine metabolites for both species and all routes included BP-3, BP-3-glucuronide, 2,4-dihydroxybenzophenone (DHB), DHB-glucuronide, and DHB-sulfates, and novel minor dihydroxy metabolites including 2,5-dihydroxy-4-methoxybenzophenone. • *In vitro* hepatic metabolism in mice differed from human and in vivo metabolism especially for phase II conjugates.
Nakagawa and Suzuki, 2002	• The metabolism and cytotoxicity of 2-hydroxy-4-methoxybenzophenone (HMB, BP-3) in **isolated rat hepatocytes** and the xenoestrogenic activity of BP-3 and its metabolites in MCF-7 human breast cancer cells and an estrogen receptor competitive binding assay have been studied, respectively.

Table VIII.A3: Skin sensitisation

Reference	Named in	Findings
CWDF, 2005	ECHA dissemination, 2021; SCCP, 2006; SCCP, 2008; SCCS, 2021	• BP-3 dissolved in DMF was assessed for its possible contact allergenic potential in the murine local lymph node assay (LLNA) in female CBA/CaOlaHsd mice (LLNA test, guideline test with GLP). • Mice ears were topically treated with the test item in concentrations of 0 (control), 12.5, 25 and 50% for three consecutive days. Five days after the first topical application the mice were injected intravenously with 3H-methyl thymidine. The Stimulation Indices (S.I.) of 1.64, 1.33 and 1.61 were determined with the test item at concentrations of 12.5, 25 and 50% (w/v) in DMF, respectively, and thus, were clearly below the limit criterium of a S.I. of 3.
	SCCS, 2021	• A supporting guinea pig maximisation test performed in 1991 also showed that the substance is not considered skin sensitizing
Beleznay et al., 2014	SCCS, 2021	• A patch test database was searched for positive patch test reactions to benzophenone-3. Charts were also reviewed for those who were further tested to the sunscreen series. Twenty-three of the 1,527 patients seen were tested to the sunscreen series. Of these, only 4 patients had a positive reaction to a sunscreen chemical or to the product they were using. In addition, 8 of the 1,527 patients who had no specific history of sunscreen allergy reacted to benzophenone-3. ACD to sunscreen was found to be very uncommon (0.8%). Other final diagnoses included ACD to excipients such as fragrances or preservatives and suspected photosensitive disorders.
Hanson and Warshaw, 2015	SCCS, 2021	• The authors report on two cases of ACD (30 years old man; 31 years old woman).
Haylett et al., 2014	SCCS, 2021	• To assess the frequency of PA and contact allergy (CA) to sunscreens in children aged < 18 years undergoing investigation for suspected photosensitivity. • Retrospective analysis of data on children who underwent photopatch testing to a standard series of nine ultraviolet (UV) filters and to sunscreen products in a single photoinvestigation centre (2000–11). Duplicate series of UV filters and the children's own sunscreen products were applied to the back, with readings taken at sample removal, and at 24 and 48 h after 5 J cm−2 UVA exposure of one series. The analysis comprised 157 children (aged 3–17 years, 69 male and 88 female). In total 10 children (6·4%) showed positive photopatch responses to UV filters and/or their sunscreen products (4·5% to UV filters, 5·7% to their sunscreen products). The responsible UV filters most often identified were benzophenone-3 and octyl methoxycinnamate. Additionally, CA reactions were observed in nine children (5·7%), with 16 children (10·2%) showing PA and/or CA to UV filters and/or sunscreen products.
Spiewak, 2013	SCCS, 2021	• This prospective study involved 1000 consecutive, first-referred dermatology outpatients. All patients with a history of dermatitis induced or aggravated by exposure to light were qualified for photopatch testing. In the study group, 36 (3.6%; 95%CI: 2.4-4.8%) persons required photopatch testing based on their clinical symptoms.

Reference	Named in	Findings
		Five patients were positive to at least one organic sunscreen, most frequently to benzophenone-3 (2 patients).
Subiabre-Ferrer et al., 2019	SCCS, 2021	• A retrospective analysis of 116 patients tested with the European photopatch test series between 2014 and 2016 was performed. Fifty-five positive photopatch test reactions in 25 subjects were recorded, most commonly caused by the topical non-steroidal anti-inflammatory drugs ketoprofen, dexketoprofen, and etofenomate. Organic ultraviolet (UV) absorbers constituted the second main category of agents eliciting positive photopatch test reactions. Among UV absorbers, benozophenone-3 and octocrylene were the most frequent photoallergens.
Valbuena Mesa & Hoyos Jiménez, 2016	SCCS, 2021	• One hundred patients investigated for possible photoallergic contact dermatitis were enrolled in the study. They were photopatch tested with a selected group of allergens, and occluded for 48 hours; the duplicate right-hand panel was irradiated with 5 J/cm2 ultraviolet (UV)A. The readings were performed on day (D)2, D4, and D6, in accordance with the guidelines of the ICDRG, and the relevance was evaluated with the COADEX system. There were a total of 20 photopatch reactions in 15 patients, 95% of which were caused by UV filters, most frequently benzophenone-3 (55%). Eight of the positive reactions (53.3%) appeared on D6.
Warshaw et al., 2013	SCCS, 2021	• A cross-sectional analysis of patients patch tested by the North American Contact Dermatitis Group between 2001 and 2010 was performed. Of 23,908 patients patch tested, 219 (0.9%) had sunscreen coded as an allergen source. Patients who were male, with occupational dermatitis, or older (older than 40 years) had significantly lower rates of allergic reactions to sunscreens; the most commonly affected areas were the face and exposed sites ($P < 0.0001$). The top 3 most frequent allergens in sunscreens were benzophenone-3 (70.2% for 10% concentration, 64.4% for 3% concentration), DL-alpha-tocopherol (4.8%), and fragrance mix I (4.0%). Less than 40% of positive patch test reactions were detected by the North American Contact Dermatitis Group screening series of 65 to 70 allergens.
Gonçalo, 2013	SCCS, 2021	• In order to establish a consensus recommendation for performing photopatch testing, a photopatch test taskforce group was established under the joint umbrella of the European Society for Contact Dermatitis and the European Society for Photodermatology in 2000. After proposing the most adequate methodology in 2004 and completing a European multicentre photopatch test study in 2011, this taskforce is recommending a list of photoallergens that should form part of a baseline series for photopatch testing in Europe. It contains mainly ultraviolet filters and drugs, mostly non-steroidal anti-inflammatory drugs. The choice of chemicals was based on the results of a recent multicentre study, previous published cases of photoallergy, and use of the substances in the European market. It is suggested that an extended list of photoallergens should be photopatch tested in selected cases, along with patients' own products.

Reference	Named in	Findings
Tawfik & Atwater, 2019	SCCS, 2021	• Case report on anaphylactic reaction to BP-3

Table VIII.A4: Overview of relevant sub-acute and sub-chronic oral and dermal repeated dose toxicity studies with BP-3

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)/ppm	Dose unit	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings and key effects
Oral toxicity studies							
Sub-acute studies							
French, 1992 (study period: 1985-1988)	ECHA dissemination, 2021; SCCP, 2006	Rats, Fischer 344, m and f (5/sex/group)	0, 3125, 6250, 12500, 25000, 50000	ppm	14 days (2 weeks)	**NOAEL 295/311 mg/kg bw/d** (3125 ppm) for the male/female rats	No clinical observations or gross pathological changes considered to be chemical-related; increased liver weights in males and females associated with cytoplasmatic vacuolisation of hepatocytes; increased kidney weights in males (chemical-related microscopic lesions in the kidney were limited to males in the 50000 ppm-group)
French, 1992 (study period: 1985-1988)	ECHA dissemination, 2021; SCCP, 2006	Mice, B6C3F1, m and f (5/sex/group)	0, 3125, 6250, 12500, 25000, 50000	ppm	14 days (2 weeks)	**NOAEL 992/1050 mg/kg bw/d** (3125 ppm) for the male/female mice	No clinical signs attributable to BP-3 esposure; increased liver weights (dose-related) in males and females associated with cytoplasmatic vacuolisation of hepatocytes in the 6250 ppm and higher dose groups; decrease in relative right kidney weights in male mice in the top 2 dose groups
Sub-chronic studies							
Lewerenz et al.,1972 (study period: 1972)	SCCP, 2006	Rats, Wistar, m and f (12/sex/group)	0, 20, 100, 500, 1000 (diet concentrations of 0, 0.02, 0.1, 0.5, 1.0%)	mg/kg bw/d	90 days feeding study	**NOEL 100 mg/kg bw/d** for male and female rats (0.1% in feed)	Dose-related reduced body weights and body weight gains; significant deviations of the blood status in female rats, decreased absolute weights of the thymus and heart in males and females; first stages of degenerative nephritis in males and females

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)/ppm	Dose unit	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings and key effects
French, 1992 (study period: 1985-1988)	ECHA dissemination, 2021	Rats, Fischer 344, m and f (10/sex/group)	0, 3125, 6250, 12500, 25000, 50000	ppm	90 days (13 weeks) (OECD Guideline 408)	**NOAEL 429/393 mg/kg bw/d for males/ females** (6250 ppm)	Significant final bw decreases at 50000 ppm (m and f) and 25000 ppm (m); enlarged kidneys with abnormal shape and granular surface; absolute and relative right kidney weights increased in the 50000 ppm-males group and in the 25000 and 50000 ppm-females groups. Histopathologic lesions in the kidney consisting of dilatation of renal tubules, regeneration of tubule epithelial cells, papillary degeneration or necrosis, and inflammation (m and f); dose-dependant increase in absolute and relative liver weights in males and females (no histopathologic changes associated with the weight increase, but changes in activities of hepatic enzymes in serum occurred) Females: Estrous cycle length significantly increased in the 12500 and 50000 ppm-dose group. Males: Significant decrease in epididymal sperm density and non-significant increase in the percentage of abnormal sperm at 50000 ppm Key effect: Microscopic kidney lesions
French, 1992 (study period: 1985-1988)	ECHA dissemination, 2021	Mice, B6C3F1, m and f (10/sex/group)	0, 3125, 6250, 12500, 25000, 50000	ppm	90 days (13 weeks) (OECD Guideline 408	**NOAEL 5981/7579 mg/kg bw/d** (25000 ppm) for males/females	No clinical signs, and at necropsy no gross lesions related to BP-3 exposure; final body weights significantly reduced at 50000 ppm for f, and from 12500 ppm for m; dose-related increase in both absolute and relative liver weights from 6250 ppm (m and f), but microscopic changes were limited to the 25000 and 50000 ppm groups (4 m and 6 f in the top concentration group had minimal cytoplasmic vacuolization of hepatocytes, this change was also present in 3 m and 2 f from the 25000 ppm group); increase in relative kidney weight, but not dose-related; chemical-related histopathologic changes in the kidney of male mice at 50000 ppm, but not in female mice or in the lower dose groups of male mice

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)/ppm	Dose unit	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings and key effects
							Females: Estrous cycle length significantly increased at 50000 ppm Males: Sperm density decreased/abnormal sperm increased at 50000 ppm Key effect: Microscopic kidney lesions
Observations made at the 14-week interim evaluation by NTP (2020), study is a part of the 2-year toxicity study		Sprague-Dawley rats (10/sex/ group),	0 (control) or 10000	ppm	14-weeks exposure with food		At 10000 ppm: Males: bw significantly reduced, absolute and relative liver weight increased, incidence of mixed-cell cellular infiltration in liver significantly increased Females: mean bw was significantly decreased (ca. 87% of control group at 14 weeks), the absolute kidney weight was significantly decreased, and the relative liver weight was significantly increased relative to the control group. Other changes in organ weights were not considered toxicologically relevant
Dermal toxicity studies							
Sub-acute studies							
French, 1992 (1985-1988)	ECHA dissemination, 2021	Rats, Fischer 344, m and f (5/sex/group)	0, 1.25, 2.5, 5.0, 10.0, 20.0	mg/rat in acetone or lotion as vehicle	14 days (2 weeks) Exposure 5 days per week	NOAEL: 20.0 mg/rat	No changes in body weight gains, food consumption, clinical observations, necropsy findings, or by histologic examination of all tissues including skin samples from the site of application for male or female rats with BP-3 applied to the skin in either acetone or lotion vehicle. In female rats given BP-3 in lotion or in aceton, liver weights were slightly increased at the 3 highest dose levels compared to the control. In male rats given the higher doses of BP-3 in lotion, smaller increases in the liver weights were also observed. Kidney weights were minimally increased in male rats given the highest dose of BP-3 in lotion and in female rats given the highest dose

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)/ppm	Dose unit	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings and key effects
							in acetone. No discernable histopathologic changes were observed associated with the increases in liver or kidney weights.
French, 1992 (1985-1988)	ECHA dissemination, 2021	Mice, B6C3F1, m and f (5/sex/group)	0, 0.5, 1.0, 2.0, 4.0, 8.0	mg/mouse in acetone or lotion as vehicle for 5 days per week	14 days (2 weeks)	NOAEL 384/432 mg/kg bw/day for the male/female mouse (applied levels were converted to dosages, (SCCP, 2006))	No chemical-related changes in body weight gain, food consumption, clinical observations, necropsy findings or histology of skin from the site of application in male and female mice receiving BP-3 in acetone or lotion; relative kidney weights were variably increased in male mice receiving BP-3 in the acetone vehicle. In male and female mice given the higher doses of BP-3 in acetone or lotion relative liver weights were increased (i.e., at 196 and 388 mg/kg bw/day).
Okereke et al., 1995	SCCP, 2006	Rats, Sprague-Dawley (6 m/group)	0, 100	mg/kg bw/d two times per day for 28 days	28 days (4 weeks)	-	No premature death was observed; no substance-related effect on body weight, relative organ weights, haematological and clinical-chemical parametes; no histopathological abnormalities of liver, kidney and testes; no differences in skin from treated and non-treated areas
		28-days oral repeated dose toxicity study in ICR mice, with 96 6 week old ICR mice (30/group), (Jin et al., 2020) 28 days oral study, applied does: 500 nM in drinking water					Mean whole blood concentration of BP-3 in mice was 0.21 ng/mL. Decreased PBE (polar body extrusion) rate of oocytes compared to controls, percentage of mouse embryos that reached the blastocyst stage was signifcantly lower, level of histone H3K4me3 significantly decreased in BP-3 group compared to control; no difference in Kdm5a mRNA expression, Kdm5b significantly increased compared to control, expression of Kdm5c significantly lower; ROS signal and rate of early apoptosis in oocytes markedly increased, GSH significantly decreased; higher percentage of spindle defects and chromosome congression failure observed in BP-3 treated oocytes.

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)/ppm	Dose unit	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings and key effects
							ROS = reactive oxygen species; GSH = glutathione
Sub-chronic studies							
French et al., 1992	ECHA dissemination, 2021	Rats, Fischer 344, m and f (10/sex/group)	0 12.5, 25, 50, 100, 200	mg/kg bw/d	90 days (13 weeks) Exposure 5 days per week	**NOAEL** **200 mg/kg bw/d (nominal)** SCCS (2008): "this NOAEL was put forward, on the assumption that deviations without dose-response relationship and without correlated histopathological findings (e.g. the decreased reticulocyte count, increased relative kidney weight, increased platelet count and whole blood cell count) should not be taken into account."	No chemical-related changes in body weight gains, food consumption, clinical observations, reproductive system evaluations, necropsy findings, or histology of skin from the site of application. Females: Relative kidney weights increased in a non-dose-related manner in rats treated topically with 25 mg/kg or larger doses of BP-3; decreases in reticulocyte counts in animals of all dose groups at 12 weeks, increases in platelet counts in animals in the 50, 100, and 200 mg/kg dose groups at 15 days, increase in white blood cells (WBC) count produced by a lymphocytosis in the 200 mg/kg group at 12 weeks

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)/ppm	Dose unit	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings and key effects
French et al., 1992	ECHA dissemination, 2021	Mice, B6C3F1, m and f (10/sex/group)	0, 22.8, 45.5, 191.0, 182, and 364	mg/kg body weight	90 days (13 weeks		No chemical-related change in body weight gains, food consumption, clinical observations, necropsy findings (including organ weights) or histology of skin from the site of application; mild, but significant increase in relative kidney weights (m), but not dose related, no abnormal histopathologic findings in this tissue Males: Significant dose-related decrease in epididymal sperm density at all doses studied (22.75, 91.0, and 364.0 mg/kg)

Table VIII.A5: Overview of relevant oral reproductive toxicity studies with BP-3

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)	duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Oral studies						
ECHA dissemination, 2021 (study from 2005)	ECHA dissemination, 2021 (study from 2005); SCCP, 2006; SCCS, 2021	25 time-mated female Wistar rats per group (teratogenicity – rat – oral)	40, 200, 1000 mg/kg bw/day (gavage)	14 days (from days 6 - 19 post coitum)	NOAEL maternal toxicity: 200 mg/kg bw/day (actual dose received); LOAEL: 1000 mg/kg bw/day	Urine stained fur, transiently affected food consumption and body weight, and a decreased net maternal body weight gain at the high dose
					NOAEL prenatal developmental toxicity: 200 mg/kg bw/day (actual dose received); LOAEL: 1000 mg/kg bw/day	Slightly increased incidence in few skeletal variations at the high dose foetuses (All 3 dose levels had no influence on gestational parameters, no test substance-related differences were recorded for placental and fetal body weights.)
Nakamura et al (2015)	SCCS, 2021	Rats, Harlan Sprague Dawley, 7-8 female rats per group	0, 1000, 3000, 10,000, 25,000, or 50,000 ppm; calculated average consumed doses as 0, 67.9, 207.1, 670.8, 1798.3, and 3448.2 mg/kg/d	from gestion day (GD) 6 to PND 23	Developmental/reproductive toxicity, NOAEL 67.9 mg /kg bw/day (1000 ppm)	Reduction of the number of spermatocytes per seminiferous tubule in offspring

Reference	Named in	Species, study type	Dosage (mg/kg bw/d)	duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
NTP (2020)	SCCS, 2021	Rats, Sprague-Dawley, modified one generation study	0, 3000, 10000 or 30000 ppm (equivalent to 242, 725 and 3689 mg/kg bw/d)	from gestion day (GD) 6 to PND 28	maternal NOAEL: 206-478 mg/kg bw/day (3000 ppm)	Reduced body weight gain during GD 6–9 and lactation day 4–21
					developmental NOEL: 206-478 mg/kg bw/d (3000 ppm)	Impaired postnatal bodyweight performance at 10,000 ppm (660-1609 mg/kg bw/day)
Dermal studies						
Daston et al., 1993	SCCP, 2006	B6C3F1 mice; 10/group	0, 20, 100 and 400 mg/kg bw/d	8-9 weeks old at start of experiment	NOAEL: 400 mg/kg bw/d for male and female mice (highest dose applied)	No effects on body weight gain or in the measured reproductive parameters

Table VIII.A6: Mutagenicity / genotoxicity

Reference	Named in	Findings
Bonin et al., 1982	SCCP, 2006	• *In vitro* bacterial mutation assay (**Ames test**) • *Salmonella mutagenesis* assays, using the standard plate incorporation test, were conducted according to established methods (Ames et al., 1975), with the standard bacterial strains TA100, TA98, TA1535, TA1537 and TA1538. Chemicals were prepared freshly in the solvent dimethyl sulphoxide (DMSO) and a minimum of 2 plates per dose point was used. Both toxicity and true genotypic reversion of mutant colonies were carefully monitored. All chemicals were tested in the presence and absence of $9 mix, prepared from the livers of Aroclor 1254-induced male (150 - 200 g) Sprague-Dawley rats. For BP-3 negative results were reported.
Hill et al., 1980	SCCP, 2006	• *In vitro* bacterial mutation assay (**Ames test**) • Referenced in SCCP, 2006; negative results in AMES test reported
Zeiger et al., 1987	SCCP, 2006	• *In vitro* bacterial mutation assay (**Ames test**) • The results and data from the testing of 255 chemicals for mutagenicity in Salmonella are presented. All chemicals were tested under code using a preincubation modification of the Salmonella/microsome test in the absence of exogenous metabolic activation and in the presence of liver S-9 from Aroclor-induced male Sprague-Dawley rats and Syrian hamsters. BP-3 was found to be nonmutagenic in one test and or weakly mutagenic, in the other. The weak positive result for BP-3 was obtained through the use of TA97 and 30% S-9, whereas the negative result was obtained with the protocol that used 10% S-9 and TA1537.
French et al., 1992 (NTP, 1992)	SCCP, 2006; SCP, 2008; SCCS 2021; ECHA, 2021	• *In vitro* chromosome aberration test in Chinese Hamster Ovary cells: • A US National Toxicology program report (1992) briefly describes the results of cytogenetic tests with Chinese hamster ovary cells, in which Benzophenone-3-induced sister-chromatid exchanges (effective dose range 5-50µg/mL) and chromosomal aberrations (20-45µg/mL) in the presence of Aroclor 1254-induced male Sprague Dawley rat liver S9. • One trial delivered a questionable result, while another trial appeared to be clearly negative.
	ECHA dissemination, 2021; SCCS, 2021	• In SCCS (2021) a new study on Mammalian Cell Gene Mutation Test in Chinese hamster lung fibroblasts (HPRT locus) is described. BP-3 was tested in an OECD Test Guideline 476 compliant study to investigate mutagenic potential at the HPRT locus (6-thioguanine resistance) in V79 Chinese hamster lung fibroblasts. A preliminary cytotoxicity experiment was performed on cell cultures with the dose levels ranging from 8.92 to 2283 µg/mL (separated by 2-fold intervals) in the presence and absence of S9-mix. Results from the preliminary cytotoxicity test were used to select the test substance dose levels for the mutagenicity experiments. BP-3 did not induce mutations at the HPRT locus of V79 cells in the presence or absence of S9-mix (ECHA dissemination, 2021).

Reference	Named in	Findings
Majhi et al., 2020	SCCS, 2021	• Majhi et al. (2020) determined DNA damage in human breast epithelial cells, following treatment with BP-3 by immunostaining with antibodies against gamma-H2AX and 53BP1. Formation of R-loops was determined with DNA:RNA hybrid–specific S9.6 antibody. Short-term exposure to the chemicals was also studied in ovariectomized mice. Immunostaining of mouse mammary epithelium was performed to quantify R-loops and DNA damage in vivo. • The authors found that concentrations of 1 µM or 5 µM BP-3 increased DNA damage similar to that of E2 treatment in an ERα-dependent manner. BP-3 exposure caused R-loop formation in a normal epithelial cell line when ERα was introduced. R-loops and DNA damage were also detected in mammary epithelial cells of mice treated with BP-3. • SCCS (2021) concluded that the biological meaning of the results of study was not clear at the moment. Although proof of the formation of R-loops and DNA double strand breaks was provided after exposure to BP-3, it is not certain how this translates into a mutagenic hazard of BP-3.
NTP, 2020	SCCS, 2021	• Bacterial mutagenicity tests conducted in **Salmonella typhimurium** strains TA98 and TA100 as well as in Escherichia coli strain WP2 uvrA pKM101, with and without rat liver S9 were negative.

Table VIII.A7: Results of carcinogenicity study in rats (study period: 2010-2012; NTP, 2020)

Beginning on gestation day (GD) 6, groups of 42, 35, 35, and 43 F0 time-mated female Sprague Dawley rats were fed diets containing BP-3 for 39 days. Groups of 50 (1,000 and 3,000 ppm) or 60 (0 and 10,000 ppm) F1 rats per sex continued on study after weaning and were fed diets containing the same exposure concentrations for 105 weeks; 10 F1 rats per sex from the 0 and 10,000 ppm groups were evaluated at 14 weeks.

BP-3				
Doses ppm	**0**	**1000**	**3,000**	**10,000**
F1 males (mg/kg bw/ d)	**0**	**58**	**168**	**585**
Survival rates	30/50	29/50	24/50	33/50
body weights	-	-	-	~10% less than in control after week 69; 20% lower than the control group at study end
Nonneoplastic effects				
Testis, interstitial cell, hyperplasia	1/50	0/50	0/50	5/50
Neoplastic effects	none	none	none	none
Equivocal findings				
Brain: malignant meningioma	0/50	1/50	3/50	0/50
Level of evidence of carcinogenic activity	**Equivocal evidence**			
Doses ppm	**0**	**1000**	**3,000**	**10,000**
F1 females (mg/kg bw/ d)	**0**	**60**	**180**	**632**
Survival rates	30/50	33/50	34/50	26/50
body weights			~10% less than the control group after week 77	~10% less than the control group after week 17; ~16% lower than the control group after week 45; 24% lower at study end
Nonneoplastic effects				
Uterus: endometrium atypical hyperplasia	9/50	14/50	19/50	14/50
Adrenal cortex: hypertrophy, focal	24/50	42/50	39/50	27/50
Neoplastic effects	none	none	none	none
Equivocal findings				
Thyroid glan: C-cell adenoma	5/50	11/50	17/50	10/50
Uterus: stromal polyp	8/50	15/50	18/50	10/50
Level of evidence of carcinogenic activity	**Equivocal evidence**			

Table VIII.A8: Results of carcinogenicity study in mice (from NTP, 2020)

B6C3F1/N mice (50/sex/group) were fed diets containing BP-3 for 104 (females) or 105 (males) weeks.

BP-3				
Doses ppm	**0**	**1,000**	**3,000**	**10,000**
male mice (mg/kg bw/d)	0	113	339	1,207
Survival rates	30/50	29/50	24/50	33/50
body weights	-	-	-	~10% less than in control after week 17
Nonneoplastic effects				
Bone marrow: pigment	3/47	2/48	9/48	50/50
Spleen: pigment	4/48	5/50	10/49	17/50
Liver: hepatocyte, syncytial altration	2/49	39/50	45/50	48/50
Kidney: renal tubule, cytoplasmatic alteration	0/48	0/50	0/50	46/50
Neoplastic effects	none	none	none	none
Equivocal findings	none	none	none	none
Level of evidence of carcinogenic activity	No evidence			
Doses ppm	0	1,000	3,000	10,000
female mice (mg/kg bw/d)	0	109	320	1,278
Survival rates	42/50	39/50	44/50	46/50
body weights	-	-	-	~10% less than in 2ontrol after week 17
Nonneoplastic effects				
Bone marrow: pigment	6/49	0/50	0/50	50/50
Spleen: pigment	12/49	10/50	36/49	38/50
Kidney: metaplasia, osseus	0/49	1/50	3(50	5/50
Neoplastic effects	none	none	none	none
Equivocal findings	none	none	none	none
Level of evidence of carcinogenic activity	No evidence			

Table VIII.A9: Phototoxicity

Reference	Named in	Findings
Spielmann et al., 1988	SCCS, 2021	• Balb/c 3T3 mice fibroblasts were incubated for 60 minutes with several concentrations (from 100 to 10000 µg/mL) of BP-3. Subsequently, the cells were exposed for 50 minutes to a sun simulator. Neutral red uptake (NRU) was determined after 24 hours. Additionally, the so-called Mean photo effect (MPE) was calculated based on the comparison of the complete concentration response curves. Substances with MPE values ≥ 1 were considered phototoxic. • Spielmann et al. (1998) concluded that BP-3 was not phototoxic in the 3T3 NRU assay in the presence and absence fof sunlight. SCCS (2021) comments that the results indicate that BP-3 is not phototoxic.
CWG unpublished	SCCP, 2006; SCCP, 2021	• *in vitro* bacterial mutation assay • The overall conclusions of the authors was that BP-3 did not induce gene mutations by base pair changes or frameshifts in the genome of the bacterial strains used and was therefore shown to be non-photomutagenic in this Salmonella typhimurium photomutagenicity test.
	SCCP, 2006	• In SCCP (2006) an in vitro bacterial mutation assay (study year: 2004) is described (Chemie Wirtschaftsfördergungs GmbH, unpublished data). BP-3 was investigated for its potential to induce gene mutations under irradiation with artificial sunlight according to the plate incorporation test (experiment I) and the preincubation test (experiment II) using the Salmonella typhimurium strains TA1537, TA 98, TA 100, and TA 102. The test was performed in two independent experiments. Each concentration, including the controls, was tested in triplicate and concentrations of 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate in experiment I of 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate in experiment II were investigated. The test substance was dissolved in DMSO. Prior to the main experiments, the induction of toxicity and mutagenicity was investigated in a pre-experiment with all strains. The overall conclusions of the authors was that BP-3 did not induce gene mutations by base pair changes or frameshifts in the genome of the bacterial strains used and was therefore shown to be non-photomutagenic in this Salmonella typhimurium photomutagenicity test.
		• In vitro photomutagenicity: chromosome aberration test • BP-3 was tested in bacterial and mammalian test systems according to valid testing guidelines and under GLP conditions with the characterized test material. No photogenotoxic/photomutagenic potential was noted in the bacterial gene mutation assays in *Salmonella typhimurium* strains and no photoclastogenic potential was recorded in the chromosome aberration test in Chinese hamster V79 cells, both with and without irradiation. • In addition, a published screening test revealed no indication that Benzophenone-3 may cause DNA damage with or without irradiation.

Reference	Named in	Findings
		• Taken from SCCP 2006 "As far as the (photo)mutagenic/(photo)genotoxic potential of Benzophenone-3 is concerned, the presented in vitro and in vivo assays indicate that the substance does not possess (photo)mutagenic of (photo) genotoxic properties."

Table VIII.A10: Neurotoxicity

Reference	Findings
In vivo	
Pomierny et al., 2019	• No effects were observed on the discrimination index in the novel object recognition test and on short-term memory. However, rats exposed to BP-3 showed a significantly lower discrimination index in the novel location recognition test and thus weakened spatial memory. The authors remark however, that clear signs of dose response and of adversity are missing. In vitro studies
In vitro	
Broniowska et al., 2016	• BP-3 evokes necrosis in high concentrations, while at a concentration of 10−8 M, it induces apoptosis in the human neuroblastoma SH-SY5Y cell line.
Wnuk et al,. 2017	• BP-3 activates the mitochondrial pathway of apoptosis in mouse neuronal primary cultures, and this effect is connected to changes in the expression of oestrogen receptors
Krzyżanowska et al., 2018	• Prolonged exposure to BP-3, like other EDCs that affect the survival of nerve cells, may be an important risk factor for the development of neurodegenerative diseases in later life. Krzyżanowska et al., (2018) showed that exposure to BP-3 in the prenatal period and then for 2 weeks in adulthood induced the mitochondrial pathway of apoptosis in the rat frontal cortex. BP-3 also reduced ERβ and GPR30 expression in the frontal cortex and hippocampus and increased AhR levels in the frontal cortex. These data suggested that the pro-apoptotic BP-3 action can be connected to an attenuation of neuroprotective oestrogen action and also might be due to intensification of aryl hydrocarbon receptormediated apoptosis, however further more detailed studies on the role of AhR are necessary.

Table VIII.A11: Endocrine-related adverse effects: in vitro and in silico studies (only studies named in SCCS (2021) and Mustieles et al. (2023) are listed)

Reference	Named in	Findings
In vitro		
(anti-)estrogenic disrupting effects		
Kunz and Fent, 2006	SCCS, 2021; Mustieles et al., 2023	• in the recombinant yeast assay expressing the rainbow trout Era BP-3, moderate, but clear dose–response curves were found BP3 characterized by maximal responses of 43–74% • (but BP-3 did not result in a significant VTG induction at all exposure concentrations in fish) • -BP-1 was found to be between 4 and 100 times more potent than BP-3 in various reporter gene assays for estrogenic activity
Kim and Choi (2014)	SCCS, 2021	• The binding affinitiy of BP-3 to ER appears to vary depending on the ER subtype
Miller et al., 2001	Mustieles et al., 2023	• BP-1 was found to be between 4 and 100 times more potent than BP-3 in various reporter gene assays for estrogenic activity
Molina-Molina et al., 2008	SCCS, 2021; Mustieles et al., 2023	• The objective of this study was to provide a comprehensive ER activation profile of BP derivatives using ER from human and fish origin in a battery of in vitro tests, i.e., competitive binding, reporter gene based assays, vitellogenin (Vtg) induction in isolated rainbow trout hepatocytes, and proliferation based assays. The ability to induce human androgen receptor (hAR)-mediated reporter gene expression was also examined. • All BP derivatives tested **except BP-3** were full hERα and hERβ agonists (BP2>THB>BP1) and displayed a stronger activation of hERβ compared with hERα, the opposite effect to that of estradiol (E2). Unlike E2, BPs were more active in rainbow trout ERα (rtERα) than in hERα assay. • All four BP derivatives showed anti-androgenic activity (THB>BP2>BP1>BP3).
Schreurs et al., 2002, 2005	SCCS, 2021; Mustieles et al., 2023	• BP-3-induced repression of progesterone receptor transcription • BP-3 was found to be a full ER alpha agonist in transfected HEK293 cells with hERa and hERb genes. BP-3 was also able to activate transcription through ERb to significant levels. • In HEK293 cells, the EC50 value of transactivation for hERα was one order of magnitude lower than for hERβ
Blair et al., 2000	SCCS, 2021; Mustieles et al., 2023	• BP-3 was determined to be an ER non-binder in a rat uterine cytosolic ER competitive binding assay (IC50 >1.00 x 10^{-4} M).

Reference	Named in	Findings
Schlecht et al., 2004	SCCS, 2021; Mustieles et al., 2023	• The study concluded that BP-3 was devoid of any uterotrophic effect but inhibited ERα transcript expression and also mildly inhibited ERβ gene expression in the uterus. The study concluded that BP-3 exerts effects which are not covered by the uterotrophic assay, but might prove to be adverse. Effects mediated via AhR and ERR1 in these organs are unknown, so the physiological relevance of the findings remains to be established. In brief, unlike BP2, an estrogen-like effect was not observed for BP-3, although regulatory effects on receptor expression were observed indicating potential endocrine disruption that is not assessed by 'classical' estrogenic markers.
Watanabe et al., 2015	SCCS, 2021; Mustieles et al., 2023	• When BP-3 was incubated with rat liver microsomes in the presence of NADPH, 2,4,5-trihydroxybenzophenone (2,4,5-triOH BP) and 3-hydroxylated BP-3 (3-OH BP-3) were newly identified as metabolites, together with previously detected metabolites 5-hydroxylated BP-3 (5-OH BP-3), a 4-desmethylated metabolite (2,4-diOH BP) and 2,3,4-trihydroxybenzophenone (2,3,4-triOH BP). In studies with recombinant rat cytochrome P450, 3-OH BP-3 and 2,4,5-triOH BP were mainly formed by CYP1A1. BP-3 was also metabolized by human liver microsomes and CYP isoforms. In estrogen reporter (ER) assays using estrogen-responsive CHO cells, 2,4-diOH BP exhibited stronger estrogenic activity, 2,3,4-triOH BP exhibited similar activity, and 5-OH BP-3, 2,4,5-triOH BP and 3-OH BP-3 showed lower activity as compared to BP-3. Structural requirements for activity were investigated in a series of 14 BP-3 derivatives. When BP-3 was incubated with liver microsomes from untreated rats or phenobarbital-, 3-methylcholanthrene-, or acetone-treated rats in the presence of NADPH, estrogenic activity was increased. However, liver microsomes from dexamethasone-treated rats showed decreased estrogenic activity due to formation of inactive 5-OH BP-3 and reduced formation of active 2,4-diOH BP. Anti-androgenic activity of BP-3 was decreased after incubation with liver microsomes. • -BP-1 was found to be between 4 and 100 times more potent than BP-3 in various reporter gene assays for estrogenic activity • BP-3 did not show any induction of proliferation in MCF-7 breast cancer cells and estrogen-responsive CHO cells at concentrations of the order of micromolar
Kerdivel et al., 2013	SCCS, 2021; Mustieles et al., 2023	• BP-3 did not show any induction of proliferation in MCF-7 breast cancer cells and estrogen-responsive CHO cells at concentrations of the order of micromolar
Nakagawa and Suzuki, 2002	SCCS, 2021; Mustieles et al., 2023	• BP-3- can be converted into at least three metabolites that exhibit estrogenic potencies, BP1, BP8 and THB
Jeon et al., 2008	SCCS, 2021; Mustieles et al., 2023	• BP-3- can be converted into at least three metabolites that exhibit estrogenic potencies, BP1, BP8 and THB

Reference	Named in	Findings
Alamer and Darbre, 2018	SCCS, 2021; Mustieles et al., 2023	• long-term exposure to the UV filters BP-3 at 10^{-7} M following 21 weeks exposure can increase the migratory and invasive properties of two human breast cancer cell lines in vitro. Increased migration was observed following exposure to these UV filters in both oestrogen responsive (MCF-7) and oestrogen unresponsive (MDA-MB-231) human breast cancer cells, implying that their ability to increase cell motility is not confined to oestrogen-responsive cells. However, despite their oestrogenic activity in gene expression assays, BP-3 was not able to increase MCF-7 proliferation
Majhi et al. (2020)	SCCS, 2021; Mustieles et al., 2023	• The results showed that in T47D cells, treatment with BP-3 at 1 µM or 5 µM increased γ-H2AX intensity in comparison with the control ($p < 0.0001$), but no dose-dependent increase was observed (1µM vs 5 µM BP-3). In MCF-7 cells, a dose-dependent increase in nuclear γ-H2AX intensity was observed after treatment with BP-3 in the range of 1–30 µM. BP-3 treatment (1–5 µM) showed increased nuclear 53BP1 intensity over control in both T47D and MCF-7, but only MCF-7 showed dose-dependent increase. The effect on γ-H2AX was however contrasted with the mRNA expression of estrogen-responsive gene TFF1, as responses to 5 µM BP-3 did not differ significantly from the control. In comparison, treatment with 10 nM E2 stimulated a 13.1-fold increase in the expression of TFF1. Based on the measures of transcriptional activity in MCF7 human breast cancer cell lines, the authors concluded that typical exposures to BP-3 appear to pose a minimal risk for breast cancer through ER-mediated transcriptional activation of target genes. • In T47DKBluc cells, which harbour an integrated ERE-luciferase reporter, BP-3 showed a lowest-observed-effect at 5 µM with transactivation increasing to a maximum 37% relative transactivation activity (RTA) in comparison with 10 nM E2. • In T47D and MCF-7 cell lines, the expression of endogenous ER target genes - AREG and PGR - was also quantified. Treatment with BP-3 at 1 µM resulted in no significant changes in mRNA expression of AREG and PGR, a concentration that led to significant increases in DNA damage in both T47D and MCF7 cells. • In T47D cells, proliferation induced by BP-3 was also compared with control treatment. BP-3 had only marginal effect at 5 or 50 µM. Low concentrations of BP-3 only marginally increased cell numbers in comparison with control.
(anti)-androgenic disrupting effects		
Ma et al., 2003	SCCS, 2021; Mustieles et al., 2023	• BP-3 showed no agonistic activity towards AR
Schreurs et al., 2004	SCCS, 2021; Mustieles et al., 2023	• BP-3 exhibited anti-androgenic activity in cell-based bioassays

Reference	Named in	Findings
Suzuki et al., 2005	SCCS, 2021; Mustieles et al., 2023	• BP-3 exhibited androgenic activity in various cell-based assays
Kunz und Fent, 2006	SCCS, 2021; Mustieles et al., 2023	• -BP-3 exhibited androgenic activity in various cell-based assays
Molina-Molina et al., 2008	SCCS, 2021; Mustieles et al., 2023	• BP-3 exhibited androgenic activity in various cell-based assays
Other endocrine MoA		
Schreurs et., 2004	SCCS, 2021; Mustieles et al., al 2023	• BP-3 exhibited disrupting effects towards progesterone (PR)
Schmutzler et al., 2007	SCCS, 2021; Mustieles et al., 2023	• Although BP-3 did not activate PR, it is the antagonist of PR as revealed by PR CALUX1 bioassay. As evaluated by an in vitro reporter system containing a duplicated thyroid hormone response element of the HLA-DR4 serotype, BP-3 can induce luciferase activity, showing agonistic activity toward THR
Heneweer et al., 2005	SCCS, 2021; Mustieles et al., **al** 2023	• increase in estrogen-regulated pS2 gene transcription in MCF-7 cells at low concentrations, both for BP-3 and BP-1, presenting EC50 values of 0.5 and 0.12 µM
Hofmann et al., 2009	SCCS, 2021; Mustieles et al., 2023	• BP-3 has been identified as a thyroid receptor agonist showing slight agonistic effects at 1 µM
Jin et al., 2020	Mustieles et al., 2023	• BP-3 inhibited mouse oocyte maturation and development to the blastocyst stage at 0.5 µM
Lee et al., 2018	SCCS, 2021;	• Results of Schmutzler (2007) (agonistic activity) are supported by a concomitant effect on expression of thyroid hormone regulated genes at 10 µM in rat pituitary (GH3) cells

Reference	Named in	Findings
	Mustieles et al., 2023	
Rehfeld et al. (2016, 2018)	Mustieles et al., 2023	• BP-3 directly affects the motiliy of human sperm cells in vitro, showing an EC50 of 4.7 µM
Santamaria et al., 2019	SCCS, 2021; Mustieles et al., 2023	• BP-3 disrupted rat follicular cell population and oocyte number at a lowest observed effect concentration (LOEC) of 6 nM
***In silico* modelling**		
Hong et al., 2016	SCCS, 2021	• this in silico modelling study used consensus classification modelling that classified substances into ER binders or non-binders based on two Decision Forest (DF) models built using two different training datasets. The DF models were validated using five-fold cross validations and external chemicals but did not take into account of possible metabolic activation/deactivation of the compounds. The models predicted BP-3 **as ER non-binder**.
Sosnovacvá et al., 2016	SCCS, 2021	• in silico assessment using QSAR Toolbox to predict more than 900 compounds from the list of food contact materials for potential to bind on ER. • The study also considered molecular weight and partition coefficient of the evaluated compounds and predicted BP-3 to have a **strong binding affinity for the ER binding site.**

Table VIII.A12: Endocrine-related adverse effects: *in vivo* studies

Reference	Named in	Findings
Schlumpf et al., 2001	SCCS, 2021; Mustieles et al., 2023	• weak activity for BP-3 with immature Long-Evans rats
NTP, 2020	SCCS, 2021; Mustieles et al., 2023	• no uterotrophic response was observed in ovariectomized rats when tested up to 1 g/kg
Kunz et al., 2006	SCCS, 2021; Mustieles et al., 2023	• In fathead minnows BP-3 did not show any induction potential of vitellogenin
Schlecht et al., 2004	SCCS, 2021; Mustieles et al., 2023	• The effects of BP-3 on the expression pattern of the estrogen receptors ERα and ERβ, the estrogen-related receptor-1 (ERR1), and the aryl hydrocarbon receptor (AhR) were investigated in adult ovariectomized rats. After a five day treatment with the chemicals BP-2 and BP-3 in two different dosages with a 17β-estradiol and a negative control group, the expression of the four receptors ERα, ERβ, ERR1 and AhR mRNA was measured in the pituitary, uterus and thyroid. Additionally, some "classical" markers of estrogenic action have been tested. • There was a significant increase in uterine weight in both treatment groups of BP-2 comparable to the effect of estradiol, while in the BP-3 treatment groups, no effect was seen. • BP-3 which was devoid of any uterotrophic effect, inhibited ERα transcript expression and also mildly the ERβ gene expression in the uterus. Whether these effects are of functional significance for the uterus remains unknown at present and deserves further investigation.
Majhi et al. (2020)	SCCS, 2021; Mustieles et al., 2023	• in vivo study in ovariectomised BALB/c mice, administered orally with BP-3 (3,000 µg/kg/d) and 17β-estradiol (250 µg/kg/d) • The nuclear c-H2AX intensity in the mammary gland of both BP-3 and 17β-estradiol treated animals was noted to be significantly higher than the animals treated with vehicle control. • However, whilst 17β-estradiol led to stimulated proliferation of mammary epithelium and increased uterine weight, no significant effects were observed for BP-3.

Reference	Named in	Findings
Nakamura et al., 2015	SCCS, 2021; Mustieles et al., 2023	• determined the effects of maternal and lactational exposure to BP-3 on development and reproductive organs of offspring of time-mated female Harlan Sprague-Dawley rats • In male offspring at PND23: ↓ AGD in male at 50 000 ppm; ↓ spermatocytes per seminiferous tubules at 3000 ppm and higher
Laplante et al., 2018	SCCS, 2021; Mustieles et al., 2023	• Acute study: No effect on uterus weight at 3000 µg/kg/d, significant but modest ↑ in the expression of ERα. • Chronic study: No effects on the number of pups, only modest non-significant change in length of gestation. No effect on the ductal density at 3000 and 30 µg/kg/d, â ductal densities of mammary glands in 212 µg/kg/d females. More epithelium in mammary glands at 212 µg/kg /d or 3000 µg/kg/d, no difference in 30 µg/kg/d group. The expression of Ki67 ↑ in the BP-3 treated females. No difference in the expression of Esr-1 in mammary epithelium, PR mRNA expression, or ER beta-mediated gene expression. • The SCCS has noted that the results presented in the study are often in contradiction with the authors' conclusions in regard to dose response, non-monotonic dose response, estrogen and progesterone time of response etc, and do not demonstrate a clear correlation between the treatment and the measured parameters.
Matouskova et al., 2020	SCCS, 2021; Mustieles et al., 2023	• No effect of BP-3 in males on bw, ↓ AGI in 30 µg and 212 µg male groups at PND21 and in puberty, but no differences in adult males, at PND 21 ↓ size of the mammary gland epithelium in males but no difference at puberty. In females, no effect on bw at PND21 but 212 and 3000 µg/kg/day females were heavier at puberty. No effect on AGI in females on PND21, a ↓ in AGI 212 µg/kg/day females at puberty but no effect in adulthood. • Modest ↑ in ductal areas of mammary glands in females at PND21. At puberty, the females exposed to 30 and 3000 µg BP-3/kg/ day had a modest increase in the size of the ductal tree and a significant increase in the extent of growth into the mammary fat pad. • A ↓ in cells positive for ERα in mammary gland of BP-3 exposed females only at 30 or 212 µg/kg/day, no differences in expression of progesterone receptor at PND21 or adulthood. A ↓ in 212 µg BP-3/kg/ day females at puberty, expression of Ki67 ↓ only in females from 30 µg/kg/day group. • The SCCS has noted that the results presented in the study are often in contradiction with the authors' conclusions in regard to dose response, non-monotonic dose response, estrogen and progesterone time of response etc, and do not demonstrate a clear correlation between the treatment and the measured parameters.
Santamaria et al., 2020	SCCS, 2021; Mustieles et al., 2023	• After the first pregnancy: No differences implantation areas at GD5, GD8, GD10 and GD12; no effects on the number of implantations, abortions, and abortion rates; fetal weight ↓ at GD14; 16.13% of foetuses affected by intrauterine growth restriction; altered pattern in maternal arteria uterine at GD10; male offspring from BP-3 exposed mice lighter

Reference	Named in	Findings
		at PND4 and from PND10 onwards; females of BP-3 exposed mice showed decreased weight at PND1, PND10 and PND13 (recovered from PND16 onwards). • The sex ratio of the offspring modified with more females than males. • After the second pregnancy: No effect on implantations and abortion numbers; placenta weights ↓at GD14 but not at GD10; no difference in fetal weight or fetus/placenta index; no modification of offspring weight curves; modified sex ratio with more females than males in the offspring of BP-3 treated mice. • The SCCS considers that whilst this study indicates a potential reproductive effects of BP-in mice, the observations need to be consolidated through further research, and for the time being it does not provide any clear scientific evidence that can be used in safety assessment of BP-3.

Table VIII.A13: Overview of short-term and mechanistic studies (modified and extended after Mustieles et al., 2023)

Reference; Study design	Named in	Species,	Dosage (mg/kg bw/d)	Administration, duration	Effect target	NOAEL / LOAEL (mg/kg bw/d)	Findings
Kariagina et al., 2020; uterotrophic assay, ovariectomized		BALB/c mice, females	0.7, 7, 70 mg/kg/day	5 days; 7 or 14 weeks old; diet (for dose-response oral gavage);	mammary gland proliferation	Effect at: 70 mg/kg/day	A diet high in saturated animal fat (HFD) plus BP-3 showed higher proliferation in response to E2 than did mice fed HFD alone.
LaPlante et al., 2018; uterotrophic assay, ovariectomized		BALB/c mice, females	3 mg/kg/day	4 days; 7-9 weeks old.	uterus weight	-	uterus weight
					gene expression in uterus	3	ERalpha mRNA significantly increased (70%) in BP-3 exposed mice (N=?)
					mammary gland histology	-	BP-3 did not alter morphology or proliferation in mammary gland. (N =?)
Majhi et al., 2020; uterotrophic assay, ovariectomized		BALB/c mice, females	3 mg/kg/day	4 days; 9 weeks old	ER-dependent R-loops and target genes, proliferation, and DNA damage in mammary gland	3	In the mammary epithelial cells nuclear S9.6 staining (as a measure of R-loops) was increased. DNA damage was increased measured by higher nuclear γ-H2AX intensity in the mammary gland. BP-3 exposure had not effects on proliferation marker Ki67 or transcriptional activation of ER-target genes (Areg and Pgr).
					Uterus weight		No effect
NTP, 2019; uterotrophic assay, ovariectomized		Sprague Dawley rats, females	320, 1000 mg/kg/day	3 days; 8 weeks old; oral gavage	Uterus weight		No effect on wet or blotted uttering weight (N = 8). No effect on body weight gain, but significantly lower body weight gain in high dose group.

Reference; Study design	Named in	Species,	Dosage (mg/kg bw/d)	Administration, duration	Effect target	NOAEL / LOAEL (mg/kg bw/d)	Findings
NTP, 2019; Hershberger assay		Sprague Dawley rats, Castrated, males	320, 1000 mg/kg/day	10 days; 8 weeks old; oral gavage	Reproductive organ weight; androgen antagonist assessment	-	No significant effects (N = 7-8)
					Reproductive organ weight; androgen antagonist assessment	1000	Co-administration of testosterone propionate and BP-3 resulted in significantly reduced glans penis and ventral protstate weight. However, bw at day 1 and bw gain was also significantly lower in this group. When analysing the organ weight with bw as co-variate the results are not statistically significant (N= 7-8).
Ohta et al., 2012; uterotrophic assay, ovariectomized		C57BL/6J mice, female	30, 100, 300, 1000 mg/kg/day	7 days; 8 weeks old Sc or oral gavage	Uterus weight	-	No effect on absolute or relative wet and blotted uterine weight (n=6); no effects were observed concerning clinical signs and body weights The authors also tested BP-1 and found that at 1000 mg/kg both relative and absolute wet and blotted uterine weights were significantly increased. When BP-1 was dosed sc anti-androgenic effects were observed from 300 mg/kg by reducing 17α-ethynyl estradiol induced increases in uterine weight. No effects were reported concerning clinical signs and body weights

Reference; Study design	Named in	Species,	Dosage (mg/kg bw/d)	Administration, duration	Effect target	NOAEL / LOAEL (mg/kg bw/d)	Findings
Robinson et al., 1994; Single administraton or repeated dose		Sprague dawley rats, Male and female	0.5; 1.67; 5 mg/kg/day	1 or 5 days; oral gavage	Chromosome abberations	-	Chromosomal abberations in rat bone marrow cells were not affected
Schlecht et al., 2004; uterotrophic assay, ovariectomized		Sprague dawley rat, female	250, 1000 mg/kg/day	5 days; adult rats; oral gavage	Uterus weight		No effects observed
						250	At 250 mg/kg/day ERβm RNA was significantly decreased in the uterus; At both 250 and 1000 mg/kg/day ERα mRNA expression in the pituitary was significantly decreased. After 1000 mg/kg/day AhR expression in pituitary decreased and ERR1 expression in thyroid decreased.
							Gene expression in pituitary, thyroid and uterus
Schlumpf et al., 2001; uterotrophic assay, ovariectomized		Long-Evans rats, female	611, 937, 1525 mg/kg/day	4 days; PND21-25; feed	Uterus weight	1525	At 125 mg/kg/day a significant increase in uterine wet weight was observed (N = 5-9)
Suzuki et al., 2005; uterotrophic assay, ovariectomized		F344-rats, female	20, 100, 500 mg/kg/day	3 days; 9 weeks old; Ip	Uterus weight		No effects on uterine weight observed. BP-1 exposure was also investigated. At 500 mg/kg/day slight but significant increase in uterine weight was observed (N = 6):

Reference; Study design	Named in	Species,	Dosage (mg/kg bw/d)	Administration, duration	Effect target	NOAEL / LOAEL (mg/kg bw/d)	Findings
Wnuk et al., 2018; Gestational exposure followed by 7 day neocortical primary cultures		Albino Swiss mice, male and female	50 mg/kg/day	10 days, GD7-16; sc	Neuron gene and protein expression and epigenetics in cells from male and female offspring	50	BP-3 induced apoptosis, ausred neurotoxicity and decreased ERα and β expression levels in neocortical cells of embryonic offspring. BP-3 inhibited globel DNA methylation and caused specific hypomethelation of the genes Gper1 and Bax, an effect that was accompanied by increased mRNA and protein expression levels. Furhtermore, BP-3 caused hypermethylation of the genes Esr1, Esr2 and Bcl2, which could explain reduced mRNA and protein levels of the extrogen receptors.
Wnuk et al., 2019; Gestational exposure followed by 7 day neocortical primary cultures					Neuron gene and protein expression and epigenetics in cells from male and female offspring	50	BP-3 impaired autophagy in terms of BECLIN-1, MAP1LC3B, autophagsomes, and autophagyrelated factors; disrupted levels of retinoid X receptors (RXRs) and peroxisome proliferator-activated receptor gamma (PPARγ); altered epigenic stauts (attenuated HDAC and sirtuin activities) inhibited post-translational modifications in terms of global sumoylation; and dysregulated expression of neurogenesis-and neurotransmitter-related genes as well as miRNAs involved in pathologies of the nervous system.

Table VIII.A14: Relevant epidemiological studies: endpoints and associations (modified after Mustieles et al., (2023))

Reference; study type/	Participants	Findings
Birth outcomes (n =14); thereof maternal exposure and birth outcomes (mother-child cohorts) (n = 8)		
Wolff et al., 2008; Mother-child cohort (USA)	404 pregnant women from the Environment and Children's Health Study	Urinary BP-3 concentrations were significantly associated with higher birth weights in male neonates, lower birth weights in female neonates
Philippat et al, 2012; Mother-son cohort (France)	191 pregnant women from the EDEN cohort; only infant males included in study)	Urinary BP-3 concentrations were associated with an increased birth weight and increased head circumference in all newborns.
Philippat et al., 2019; Mother-son cohort (France)	473 pregnant women from the EDEN cohort; only infant males included in study)	BP-3 was positively associated with placental weight.
Tang et al., 2013; Mother-child cohort (China)	567 pregnant women	BP-3 concentrations were positively associated with decreased gestational age (especially among boys), but not with birth weight or birth length.
Ferguson et al., 2018; Nested case-control mother-child cohort (USA)	82 pregnant women from hetLIFECODES study (130 preterm birth cases and 352 controls)	Urinary BP-3 concentrations were not associated with birth weight z-scores among the whole cohort in adjusted models; an interaction by infant sex was reported for abdominal circumference and fetal weight. In adjusted models prenatal urinary BP-3 concentrations were significantly associated with a decreased abdominal circumference among boys.
Krause et al., 2018; Prospective study (Denmark)	157 pregnant women	No significant associations were observed between matertnal BP-1 and BP-3 concentrations and birth outcomes in males and female fetus. In the medium exposure group, serum 4-hydroxy-benzophenone (4OHBP) concentrations were associated with lower birth weight, head circumference and abdominal circumference.
Messerlian et al., 2018; Prospective preconception corhort of subfertile couples (USA)	346 prospective mothers and 184 fathers from the EARTH study	Paternal preconception BP-3 concentrations were positively and significantly associated with birth weight. This association remained after further adjustment for maternal prenatal BP-3 concentrations (with a stronger association in boys than in girls).

Reference; study type/	Participants	Findings
Mustieles et al., 2020; Prospective preconception corhort of subfertile couples (USA)	417 prospective mothers and 229 fathers from the EARTH study	Both paternal and maternal preconception BP-3 exposure was not associated with the risk of preterm birth. However, a trend towards lower preterm birth risk was reported.
Aker et al., 2019; Mother-child cohort (Mexico)	749 pregnant women	High maternal mean prenatal urinary BP-3 concentrations were associated with significantly higher gestational gage, and a lower risk of preterm birth. BP-3 was consistently associated with increases in gestational age at all time points after stratification by study visit. Marginally significant positive associations were found between maternal mean prenatal urinary BP-3 concentrations and birth weight z-scores and with the risk of being born large for gestational age. In the reported associations no differences by fetal sex were observed.
Long et al, 2019; Mother-child chort (China)	847 pregnant women	Maternal urinary concentrations of BP-1 and 4-hydroxy-benzophenone (4OHBP) were significantly associated with birth length in all infants in the first trimester. Differences between the sexes were observed: in girls, both maternal BP-1 and BP-3 concentrations were significantly associated with decreases in birth weight and birth length.
Chen et al., 2013; Case-control study (China)	70 case couples, 180 control couples	Both paternal and maternal urinary BP-3 concentrations were not associated with spontaneous abortion.
Bae et al., 2016; Longitudinal preconception LIFE cohort (USA)	220 preconception couples	Paternal preconceptions as well as couple preconception BP-1 and BP-3 exposure tended to be associated with a borderline excess in male births, the associatons were not significant, however.
Fernandez et al, 2016; Case-control study (Spain)	28 cases, 51 controls	Due to the low frequency of detected (i.e., 4% of the placenta samples) no analyses could be performed.
Chevrier et al., 2012; Nested case-control study (France)	69 cases, 206 controls	There were no significant differences in maternal BP-3 concentrations between cases and controls.

Reference; study type/	Participants	Findings
Fertility and reproductive outcomes (16)		
Female related reproductive endpoints (n = 8)		
Buck Louis et al., 2014; Longitudinal preconception LIFE cohort (USA)	396 males and 411 females (>18 years old)	Maternal and paternal BP-1 and BP-3 concentrations were not associated with fecundity.
Minguez-Alarcón et al., 2019; Prospective cohort study in subfertile couples (USA)	304 women (18-45 years)	No associations were found between urinary BP-3 concentrations and follicle stiumulating hormone (FSH) serum levels or total/mature oocyte yield among fertile women seeking fertility care. For women who reported spending time outdoors performig moderate/heavy work, associations were found between increasing quartiles of BP-3 and adjusted probabilities of implantation, clinical pregnancy and live birth. Further, positive associations were also found for urinary BP-3 with self-reported sunscreen use, physical activity and time spent on outdoor work.
Arya et al., 2020; NHANES cross-sectional study (USA)	789 women (18-45 years old)	The cluster of BP-3, Triclosan (TCS) and bisphenol A (BPA) was significantly associated with a history of infertility. However, when analysed individually, no associations were found between each compound and infertility.
Pollack et al., 2018; Repeated measures clinic study (USA)	143 menopausal women (18-44 years)	Creatinine-adjusted BP-1 and BP-3 concentrations were associated with increased progesterone and with decreased 17-β-estradiol (E2), follicle stimulating hormone (FSH), and luteinizing hormone (LH) serum levels.
Pollack et al., 2015; ENDO study (USA)	495 menstruating women (18-44 years old)	GM creatine-corrected BP-1 and BP-3 concentrations were significantly higher in women with fibroids. In adjusted models this association was only marginally significant.
Kunisue et al., 2012; ENDO study (USA)	622 menstruating women (18-44 years old)	In the operative cohort, there was a signifant trend observed between BP-1 and the odds of endometriosis. Also, BP-3 exposure tended to be associated with increased risk of endometriosis, the associations were more evident with BP-1.

Reference; study type/	Participants	Findings
Peinado et al., 2020; Case-control study in ENDEA study (Spain)	124 women (35 cases aged 38; 89 controls aged 36 years)	The risk of endometriosis was significantly increased in women in the second versus first tertile of BP-1 and BP-3 after adjustment for confounders.
Gu et al., 2019; Case-control study (China)	40 women with polycystic ovary syndrome (PCOS), (30.5 years); 83 women (control) (29.8 years)	BP-3 (neither detection rates nor total concentrations) was not significantly differend in women with polycystic ovary syndrome (PCOS) and controls
Parada et al., 2019; Case-control design coupled to a follow-up study in the LIBCSP cohort (USA)	711 women with breast cancer; 598 women without breast cancer (controls)	No association was observed between BP-3 and breast cancer incidence when cases and controls were compared. BP-3 was not significantly associated with total or breast-specific mortality among the cases. Strikingly, some trends towards lower risk of breast-specific mortality were observed for BP-3 (and other phenols).
Barr et al., 2018; Pilot study breast cancer (United Kingdom)	40 women undergoing mastectomy for primary breast cancer	In breast tissues, significantly more BP-3 was measured when a tumor was present for the lateral region
Male related reproductive endpoints (n = 5)		
Scinicariello and Buser, 2016; NHANES cross-sectional study (USA)	588 children (6-19 years	In male teenagers, higher urinary BP-3 concentrations were associated with significant lower total testosterone (TT) serum levels.
Adomnei et al., 2018; Cross-sectional study (Spain)	215 students (18-23 years old)	Both BP-1 and BP-3, were significantly positively associated with follicle stimulating hormone (FSH) levels. Furthermore, BP-1 was associated positively with total testosterone (TT)/17-β-estradiol (E2) and negatively with Inhibin B/FSH ratios.
Joensen et al, 2018; Cross-sectional study (Denmark)	65 men (FLG loss-of-funciton mutation cariers), 130 men (controls)	Higher urinary BP-3 concentrations were associated with higher total testosterone (TT) and 17-β-estradiol (E2) serum levels in a group carrying filaggrin gene (FLG) mutation – this was not observed in the control group.

Reference; study type/	Participants	Findings
Buck Louis et al., 2015; Longitudinal preconception LIFE cohort (USA)	413 men (>18 years old)	Urinary creatinine-adjusted BP-1 or BP-3 concentrations were not associated with seminal quality. However, associations were found with other benzophenones, e.g., BP-8.
Smarr et al., 2018; Longitudinal preconception LIFE cohort (USA)	339 male partners (>18 years old)	Both BP-1 and BP-3 were not significantly associated with poorer sperm quality, but most associations were towards this endpoint. For BP-2 a negative association with sperm quality was observed.
Buck Louis et al., 2018; Longitudinal preconception LIFE cohort (USA)	339 male partners (>18 years old)	After covariate adjustment, neither BP-1 nor BP-3 was significantly associated with fecundability. However, couple-adjusted models are suggestive of diminished fecundity.
Chen et al., 2013; Case-control study (China)	887 idiopathic infertile men, 713 fertile controls	No associations were found between BP-3 concentrations and semen quality parmeters.
Child anthropometry and puberty (n = 6)		
Xue et al., 2015; Pilot cross-sectional study (India)	49 obese and 27 non obese children (2-14 years old)	Urinary BP-3 concentrations were not significantly different in obese and non-obese children.
Buckley et al., 2016; Longitudinal birth cohort (USA)	173 mother-child pairs	Maternal prenatal urinary BP-3 concentrations were associated with lower percent fat mass, modified by child sex this association existed only in female children.
Deierlein et al., 2017; Longitudinal puberty cohort (USA)	1017 girls	BP-3 exposure was associated with lower adiposity from the age of 10 onwards; this achieved significance at the age of 12 and 13 in relation to reduced waist circumference.
Buttke et al., 2012; NAHNES cross-sectional study (USA)	440 female participants (12-16 years old)	BP-3 exposure was not associated with the age of menarche.

Reference; study type/	Participants	Findings
Oskar et al., 2020; NHANES cross-sectional study (USA)	229 female participants (12-16 years old)	A combination with lower mono-2-ethyl-hexy-phthalate (MEHP) and BP-3 tended to be associated with earlier age at menarche.
Wolff et al., 2015; Longitudinal puberty cohort (USA)	1239 girls	BP-3 concentrations in girls in the fith quintile were significantly associated with delayed breast development.
Binder et al., 2018; Longitudinal GOCS cohort (Chile)	200 girls	In girls at 8 years BP-3 exposure was significantly associated with earlier menarche (but not at 11 years).
Harley et al., 2019; Longitudinal birth cohort (USA)	338 children in the CHAMACOS birth cohort	Both prenatal and prepubertal BP-3 exposure were not associated with pubertal timing. Increasing prenatal and prepubertal concentrations were non-significantly associated with trends towards earlier menarche.
Huang et al., 2020; Longitudinal birth cohort (China)	521 children (7-15 years old)	BP-3 exposure was longitudinally and inversely associated with stages of testicular volume in boys.
Almstrup et al., 2020; Longitudinal COPENHAGEN puberty sub-cohort (Denmark)	31 boys and 20 girls	In children with intermediate levels of BP-3 a higher methylation level of the thyroid hormone receptor interactor 6 gene (TRIP6) promoter was observed compared to the control groups. (TRIP6 is mainly induced in testicular Leydig cells during puberty – in longitudinal studies promoters of TRIP6 were significantly associated with BP-3).
Thyroid hormones biomarkers (n = 7)		
Berger et al., 2018; Prospective CHAMACOS mother-child cohort (Mexico)	454 pregnant women (>18 years old) and 365 neonates	Higher urinary BP-3 concentrations were associated with lower thyroid stimulating hormone (TSH) in newborns and lower free tyroxine (FT4) and total tyroxine (TT4) serum levels in pregnant women in unadjusted models. In models adjusted for covariates the results were slightly attenuated.

Reference; study type/	Participants	Findings
Aker et al., 2016; Prospective mother-child cohort (Puerto Rico)	106 pregnant women (18-40 years old)	BP-3 concentrations were inversely associated with maternal free triiodothyronine (FT3) serum levels. At the second visit higher BP-3 concentrations were associated with greater sex hormone binding globulin (SHBG) serum levels.
Krause et al., 2018; Prospective study (Denmark)	183 pregenant women	Maternal urinary BP-1 or BP-3 concentrations were not significantly associated with serum thyroid or growth factor levels. Higher maternal serum 4-hydroxy-benzophenone (4OHBP) levels were associated with increased total triiodothyronine (TT3) and total tyroxine (TT4) levels in mothers carrying male foetuses, in mothers carrying female foetuses 4OHBP was associated with decreased free tyroxine (FT4) serum levels.
Aker et al., 2018; Nested cas-control study within longitudinal LIFECODES birth cohort (USA)	439 pregnant women (>18 years old); 116 preterm birth cases and 323 controls	Maternal urinary BP-3 concentrations were associated with all thyroid hormones at least at one visit. BP-3 was negatively associated with total triiodothyronine (TT3) levels at all visits except the second one and with free tyroxine (FT4) levels at all visits except the fourth one. At the last visit, BP-3 was also negatively associated with the triiodothyronine (T3)/thyroxine (T4) ratio. Especially at the second and third visits, BP-3 concentrations tended to be positively associated with thyroid stimulating hormone (TSH).
Przybyla et al., 2018; NHANES cross-sectional study (USA)	710 females and 850 males (>12 years old)	Urinary BP-3 concentrations were negatively associated with total tyroxine (TT4) in males. In females BP-3 tended to be positively associated with total triiodothyronine (TT3) serum concentrations – although not statistical significance was reached.
Kim et al., 2017; NHANES cross-sectional study (USA)	1829 individuals (>12 years old)	Higher urinary BP-3 concentrations were associated with decreased serum levels of total tyroxine (TT4) and free tyroxine (FT4). Further, a tendency was observed of associatons between BP-3 and decreased total triiodothyronine (TT3) and free triiodothyronine (FT3) levels, but increased thyroid stimulating hormone (TSH) levels.

Reference; study type/	Participants	Findings
Neurodevelopment (n = 5)		
Nakiwata et al., 2018; Prospective EDEN mother-child cohort (France)	425 mother-son pairs (5-6 years old)	No associations were found between prenatal BP-3 exposure and boys' verbal or performance intelligence quotient (IQ).
Philippat et al., 2017; Prospective EDEN mother-child cohort (France)	529 children (3 years old); 464 children (5 years old)	No associations were found between prenatal BP-3 exposure and child behaviour.
Guo et al., 2020; Prospective SMBCS mother-child cohort (China)	386 mother-singleton pairs	Prenatal exposure to BP-3 was associated with a higher risk of prosocial problems among all children. Urinary BP-3 concentrations were not significantly associated with thyroid function biomarkers.
Freire et al., 2020; Prospective INMA mother-child cohort (Spain)	191 mother-singleton pairs	Associations could not be determined due to the low frequencies of detection for BP-1, BP-3 and BP-8-
Jiang et al., 2019; Prospective mother-child chort (China)	478 mother-child pairs	No associations were found between maternal exposure to BP-1 and BP-3 and children's cognitive development.
Metabolic Health and bone health (n =)		
Wang et al., 220; Longitudinal birth cohort (USA)	217 pregnenat women from fertility clinics in EARTH study	Higher urinary first trimester BP-3 concentrations were associated with lower glucose levels. Further, women with higher second trimester BP-3 concentrations had lower odds of abnormal blood glucose measured at glucose load tests (GLDs).
Ward et al., 2020; NHANES cross-sectional study (USA)	8498 adults (>20 years old)	Urinary BP-3 concentrations were significantly associated with a lower diabetes risks

Reference; study type/	Participants	Findings
Li et al., 2018; Case-control study (Saudi-Arabia)	54 T2DM cases, 47 non-diabetic controls (28-68 years old)	Both, urinary BP-1 and BP-3 in the third and fourth quantiles were significantly associated with a lower diabetes risk.
Salamanca-Fernández et al., 2020a; Longitudinal adult cohort (Spain)	670 adults in a sub-sample of the Granada EPIC-Spain cohort	Especially in women, BP-1 serum concentrations showed an inverse non-significant trend with diabetes risk.
Salamanca-Fernández et al., 2020b; Longitudinal adult cohort (Spain)	670 adults in a sub-sample of the Granada EPIC-Spain cohort	Both BP-1 and BP-3 serum conentrations were not associated with incident hypertension.
Kang et al., 2019; Cross-sectionsl study (Korea)	441 women (20-45 years old)	Higher urinary creatinine-adjusted or specific gravity adjusted BP-3 and BP-1 concentrations were associated with greater albumin to creatinine ratio (ACR) values in urine in adult women.
Li et al., 2020; NHANES cross-sectional study (USA)	7114 adults (>20 years)	In adjusted models, participants with BP-3 concentrations in tertile 2 and 3 were more likely to show increased osteoarthritis (OA) prevalence compared to participants with BP-3 concentrations in tertile 1. The observed association was stronger in males and overweight patients.
Wang et al., 2020; NHANES cross-sectional study (USA)	4445 adult (>20 years); 2,267 men; 1,145 premenopausal women and 1,033 postmenopausal women	In men and postmenopausal women urinary BP-3 tended to be associated with a higher bone mineral density (BMD) of femur.
Oxidative stress and allergy/asthma (n = 7)		
Ferguson et al., 2019; Case-control study nested in longitudinal LIEFECODES birth cohort (USA)	439 pregnant women (>18 years old); 116 preterm birth cases and 323 controls.	In models which included urinary dilution as a covariate, higher urinary BP-3 concentrations were associated with higher urinary 8-hydroxy-2′-deoxyguanosine (8OHdG) and 8-isoprostane. [Oxidative stress was evaluated measuring 8OHdG for DNA damage and urinary 8-isoprostane as a marker of lipid peroxidation].

Reference; study type/	Participants	Findings
Watkins et al., 2015; **Longitudinal** PROTECT cohort (Puerto Rico)	105 pregnant women (markers of inflammation); 54 pregnant women (markers of oxidative stress); (18-40 years old)	Prenatal exposure to BP-3 was found to be associated with an increase in 8-isoprostane levels and a decrease in c-reactive protein (CRP) levels. [8-isoprostane: marker for oxidative stress; CRP: marker for inflammation]
Rocha et al., 2018; Cross-sectional study (Brazil)	300 children (6-14 years old)	Higher urinary creatinine-adjusted BP-3 concentrations were correlated with greater urinary 8-hydroxy-2-deoxy-guanosine (8OHdG) concentrations.
Asimakopoulos et al., 2016; Cross-sectional study (Saudi Arabia)	130 participants (media age: 35 years)	Both for BP-1 and BP-3 specific models were not presented. However, benzophenones were not among the chemical families more strongly associated with 8-hydroxy-2-deoxy-guanosine (8OHdG) levels.
Artacho-Cordón et al., 2019; longitudinal hospital-based GraMo cohort (Spain)	Subsample of 144 adults from GraMo cohort	Higher BP-3 concentrations in adipose tissue were associated with reduced glutathione (GSH) levels and increased glutathione peroxidase (GPx) activity.
Buckley et al., 2018; Mother-child cohort (USA)	164 mother-child pairs	Prenatal exposure to BP-3 was inversely associated with wheeze in the past 12 months in all children.
Vernet et al., 2017; Mother-child cohort (France)	587 mother-child pairs	Prenatal exposure to BP-3 was inversely associated with wheezing until the age of 5 years and a protective non-significant trend towards lower asthma risk. No associations were oberserved between prenatal exposure to BP-3 and forced expiratory volume (FEV).

Table VIII.A15: Summary of available in vivo developmental toxicity studies with BP-3 in rodents with gestational exposure (modified after Mustieles et al., 2023)

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
Chapin, 1997; Reproductive assessment of continuous breeding	CD-1 mice	Until five litters had been produced; feed	~1800, 4000, 9000 ppm	female	Maternal parameters F0		In dam weights dose-related decreases were observed (N=)
					Liver and kidney weights F0		No effects were observed (N=5)
					Litter parameters (produced by F0)		In the medium and high dose group the number of litters dropped by 222 and 23%, respectively. For animals of the high dose group cumulative days to litter were increased by 11, 9 and 7% for litters 1, 2 and 3, respectively (N=5).
					Body weights F1 females		In middle and high dose groups decreased body weight was observed .
					Fertility F1 females		There were no changes observed in the mating, pregnancy, or fertility indices, number of pups per litter or litter viability. In the middle and high dose groups, F2 pup weight adjusted for litter size was reduced by 7 and 10%. In the two highest dose groups, F1 dam weights were reduced by 7 and 15%,respectively. In all dose groups clinical signs were observed.
					Body weights F1 females		In the high dose grou body weight was reduced by 11%.
					Liver and kidney weight F1 females		In the high dose group adjusted liver and kidney weight were increased by approximately 30 and 10%.
					Estrous cylicity in F1 females		No changes in estrous cycle length were observed.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
				male	Body weight F0 males	9000	In the high dose group body weight was decreased (N=5).
					Liver and kidney weights F0		No effects were observed (N=5).
					Body weights male F1	4000	In the middle and high dose groups a decreased body weight was observed.
					Reproductive organ weights F1 males	9000	In the high dose group decreased absolute testis weight of 6% and increased epididymis weight were observed. Adjusted prostate weight was increased by approximately 30% in this group.
					Liver weight F1 males	9000	In the high dose group adjusted liver weight weas increased by 50%..
					Sperm parameters F1 males		In the high dose group no effects on epididymal sperm number, motitility, and morphology observed.
LaPlante et al., 2018; Prenatal and lactational exposure	Balb/C mice	6 weeks; GD0-LD21; Oral (by pipet)	0.03, 0.012, 3 mg/kg/day	female	Pregnancy outcomes		No effects observed on number of pups born or on pup survival. From 1.12 mg BP-3 a non-statistically significant increased gestational length was observed. No differences in dam body weights were observed (N=?)
					Mammary gland histology	3	In the highest dose group, ductul densitiy at 5 weeks after weaning was significantly lower. Ki67 expression (used as a marker of proliferation) was significantly increased (N=?).
					Mammary gland gene and protein expression	0.212	ERα gene expression was non-statistically significant increased after 5 weeks weaning compared to the contols. No effect on ERα protein expression was observed.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
							No effects were observed on progesterone receptor gene expression, but protein expression (%positive celles) was increased from 1.12 mg/kg BP-3. Further, BP-3 did not alter Wnt5a gene or protein expression ro CCAAT/enhacer-binding protein delta and TGF-b2 expression (N=?)
Krzyzanowska et al., 2018; prenatal	Sprague Dawley rats	Entire gestation (22-23 days) + PND 43-56; **dermal**	100, Twice daily; mg/kg/day	male	Protein level of apoptotic markers in brain	100	In the frontal cortex the active (but not the inactive) form of Caspase3, Bax and Bak levels were increased. BP-3 did not affect Bcl-2 and Bcl-xL. TUNEL assay revealed increase in the number of apoptotic cells after exposure to BP-3. Increased levels of the active caspase-9 from were observed and no changes in caspase -8. In the hippocampus, an increase of caspase-9 level was observed, but not in any of the other markers.
					ERα and ERβ and GPR30 mRNA and protein levels in brain	100	In the fronal cortex a decrease in Erβ mRNA and nuclear fraction protein levels and BPR30 membrane fraction protein levels were observed. In the hippocampus, Erβ mRNA and nuclear fraction protein levels were decreased in BP-3 exposed rats, and increased Erα mRNA but decreased cytosol and nuclear fraction protein levels and decreased GPR30 membrane fraction protein levels.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
					AR and AhR mRNA and protein in brain	100	At mRNA level, no effects were observed in frontal cortex or hippocampus At protein level, higher nuclear fraction AR levels were observed in BP-3 exposed males in both tissues and in frontal cortex an increased AhR cytosol fraction was observed.
					Testosterone, 17β-estradiol, progesterone and prolactin plasma concentrations	100	Reduced testosterone levels were observed in BP- exposed male rats. No effects were observed on 17β-esradiol, progesterone and prolactin levels.
					TSH, free T3 and free T4 plasma concentrations		No effects observed (N = 10).
Matouskova et al., 2020 Perinatal exposure	Balb/C mice	6 weeks, GD0-LD21; Oral (by pipet)	0.03, 0.212, 3 mg/kg/day	female	Mammary gland histology	0.03	Larger ductal areas compared to controls were observed at PND21, but no significant effects were observed for the extent of growth into the fat pad (ductal extension). In the lowest dose group, a modest (non-significant) increase in the size of the ductal tree was observed **at puberty** and a significant increase in the extent of growth into the In the high dose group a significant increase in the number of terminal end buds was observed as well as a trend towards signifance for a linear increase by dose. Between groups, there were no significant differences in total terminal end bud area, although there was a trend toward significance for a linear increase by dose.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
							In adulthood, a decreased fraction of the mammary gland comprised of ducts was observed with increasing dose. In the highest dose group, the fraction of the gland comprised of alveolar buds was increased (N=9-11).
					Mammary gland ER and PR expresssion	0.03	After 0.03 and 0.212 exposure a significant decreased fraction of ERα-positive cells was observed in adult females. At puberty, PR-positive epithelial cells were significantly decreased in the 0.212 mg group.
					Mammary gland proliferation markers	0.03	At PND21 and puberty (but not in adulthood), the fraction of proliferating cells was reduced. This was only statistically significant, however, at PND21 after 0.03 mg BP-3. A significant linear relationship between dose and Ki67 positive cells was observed at PND21 and adulthood.
				male	AGD (short)	0.03	After exposure to 0.03 and 0.212 the AGD index was shorter at PND21 and in puberty (PND32-3). No differences were observed in adult males (postnatal week 9-11). No effects on male body weights were observed.
					Mammary gland histology	0.03	At PND21 the size of the mammary gland epithelium on both sindes were significantly reduced in all dose groups, there were also fewer branching points. No effects were observed at puberty or adulshood (N=9-11).

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
Nakamura et al., 2015; Gestatational and lactationa	Sprague Dawley rats	GD6-PND23; feed	1000, 3000, 10000, 25000, 50000 ppm	female	Dam clinical signs, food consumption and body weights	2500	No clinical signs were observed in dams. Gestational body weights (GD10-20) decreased in a statistically significant dose-dependent manner in the 25000 and 50000 dose groups. At GD10-13, a derease in food consumption was observed in the two highest dose groups, for the highst dose group this was also observed at PD14-23.
					Dam liver and kidney weight	10000	In the 10000 and higher dose increased absolute and relative liver weights were observed in dams at PND23 compared to the control. In the highest dose group kidney weights were increased compared to the control.
					Dam ovary and uterus weight	1000	Only dams in the 1000 ppm dose group had increased absolute and relative ovary and uterus weights at PND23.
					Litter parameters		No effects on the number of implantation sites, resorptions at GD10, 1, and 20 were observed. Also, no effects on the number of litters with surviving pups at PND23 number or weights of live fetuses, or sex ratios at GD20 were observed.
					Body weight of female offspring from PND-1-23	25000	In the highest dose group, body weights were decreased at PND14 and 23. This was also observed in the 25000 ppm group at PND23.
					AGD		No effects on AGD normalized to body weight were observed.
					Reproductive organ weight, female offspring	25000	In the two highest dose groups, body and ovary weights were significantly decrased on PND23, but ovarian/body weight ratios were not different. In the highest dose group, uterine

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
							weight was also lower, but uterine/body weight ratios were not.
					Liver and kidney weight of female offspring	10000	In the 10,000, 25,000 and 50,000 ppm dose groups relative liver to body weight ratios were significantly increased. Kidney weights were significantly lower than in controls, but not relative to body weights.
					Ovary histology, female offspring	10000	In the 10,000 and 50,000 ppm groups, the number of antral follicles was significantly lower at PND23. No differences were observed in the number of small, medium or large follicles.
				male	Body weight of male offspring from PND1-23	25000	In the highest dose group, body weights were decreased at PND14 and 23. At PND23 this was also observed in the 25000 pppm group (N=4-8 litters).
					AGD	50000	At PND1 not effects on AGD normalized to bw were observerd. At PND23 a significantly shorter AGD was observed in the high dose group (N=4-6 litters).
					Nipple retention and testes descend		No differences were observed in nipple retention and testes descent.
					Repdroductive organ weight, male offspring	50000	On PND23 testes weights decreased in a dose-dependent manner (this was only significant however in the high dose group -absolute, not relative)
					Liver and kidney weight, male offspring	50000	No effects on liver weigths were observed. In the highest dose group absolute (but not relative) kidney weight was significantly decreased.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
					Testis histology, male offspring	3000	At doses of 3000 ppm and higher, the number of spermatocytes per seminiferous tubule was significantly reduced. No effects were observed on the number of Sertoli cells per seminiferous tubule. In the 50,000 ppm group, some seminiferous tubules contained a few or no spermatocytes. At 10,000 ppm or higher, the number of seminiferous tubules with three or more apoptotic cells (TUNEL) was significantly higher.
					Blood T3 levels, male offspring		In the 25,000 and 50,000 ppm groups T3 levels were reduced by 38% and 44%, respectively (but not statistically significant).
					Blood testosterone levels, male offspring	3000	In groups exposed to 3000 and 25,000 ppm BP-3, serum testosterone levels were significantly lower than in the controls.
Nakamura et al., 2018; Prenatal and lactational	Sprague Dawley rats	GD6-PND21, diet	300, 30000 ppm	male	Body weight	30000	In the high dose group, body weight was 22% lower at PND30.
					Reproductive organ weight	30000	In the high dose group, absolute weight of the testis, epididymis and prostate were lower at PND30 than in the controls, but relative to body weight, only the relative testis weight was significantly lower. No effects were observed on seminal vesicle weight.
					Liver & kidney weight	30000	In the high dose group, absolute liver and kidney weights were lower, decreasing in a dose-dependent manner. Relative to bw, no effects were observed.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
					Blood testosterone levels	30000	In the low and high dose groups lower testosterone levels compared to the control were observed. This was only significant, however, for the high dose group.
					testis gene expsression	30000	At PND30 microarray analyses of testes revealed 239 differentially expressed genes (DEGs) in low dose compared to control and 1159 DEGs in high dose compared to control – this was only statistically significant however, in the high dose group. DEGs included estrogen-related genes, somatic ubiquitously expressed genes, tumor suppressors, and testis/spermatogenic genes. Steroid biosynthesis, energy metabolism and apoptosis GO categories were also affected in the testis of the 30,000 ppm group.
					Prostate gene expression	30000	At PND30 microarray analyses of prostate revealed 334 differentially expressed genes (DEGs) in the low dose group and 689 in the high dose group. DEGs included genes in the cell cycle and proliferation pathway, however changes were not statistically significant.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
NTP, 2019; 2-year carcinogenicity study	Sprague Dawley	2 years, GD6 onwards; feed	0, 1000, 3000, 1000 ppm Females: 58, 168 and 585 mg/kg/bw Males: 60, 180, and 632 mg/kg/bw	female	Body weight and food consumption	10000	In the 1000 ppm group mean body weights of F1 females were 10-25 than in controls over the course of the experiment. After week 77, F1 female body weights were 10% lower in the 3000 ppm group than in females of the control group. BP-3 exposure had no effect on food consumption.
					Thyroid histopathology	3000	The incidence of thyroid gland incidence of C-cell adenoma was higher in 3000 ppm females at the end of the study (17 vs 5 in the control group)
					Uterus histopathology	3000	In the 3000 ppm females, the incidence of stromal polyps was significantly increased (18 vs 8 in the control group). Further, a significantly increased incidence of atypical endometrium hyperplasia of the uterus was observed in this group (19 vs 9 in controls); the incidence of adenocarcinoma was significantly decreased in this group.
					Adrenal histopathology	1000	In the 1000 and 3000 ppm females the incidence of focal hypertrophy in the adrenal cortex were significantly increased at the end of the 2-year study (42 and 39 vs 25 in controls)
					Pregnancy outcomes & maternal parameters	3000	In the 1000 ppm group gestation weights of dams were slightly lower than control. From 3000 ppm body weight gains from GD6-21 were slightly decreased. [during gestation doses correspond approximately to 70, 206, and 660 mg/kg/bw]. No clinical signs were observed.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
					Litter parameters	10000	No effects were observed on litter size or sex ratio. In the high dose group litter size was slightly lower on PND7 and 10.
				male	Body weight and food consumption	10000	In the 10000 ppm group, mean body weights of F1 males were 10-25% lower over the course of the study than in the controls. No effects were observed on food consumption. [doses correspond to approximately 58, 168, and 585 mg/kg body weight for males)
					Testis histopathology	10000	In 10000 ppm males the incidence of fibrinoid necrosis of the arterioles was significantly increased in the testes at the end of the 2-year study (16 in control vs 25 in high dose group), and the incidence of interstitial cell hyperplasia occurred with a positive trend (1 in control vs 5 in high dose).
NTP, 2021; Gestational and lactational exposure; dose range-finding study	Sprague Dawley rats	GD6-PND28, feed	3000, 10000, 25000, 50000 ppm	female	Pregnancy outcomes & maternal parameters	50000	No effects were observed on % pregnenat F0 or F1 females or gestation length. In the high dose group, dam body weights were significantly dereased during gestation. No clinical effects were observed. Doses correspond to 215, 695, 2086, and 6426 mg/kg/day during gestation.
					Litter parameters	10000	On PND0 no difference in litter size or sex ratio were observed. In the 10000 and 50000 ppm groups, PND1 pup weight was significantly decreased and pup weights were generally lower in the two highest dose groups during the lactation period (PND1-28). Also, the number of live pups was lower in these dose groups over the lactation period.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
NTP, 2021; Modified one-geneartion. Gestational, lactational and adult exposure	Sprague Dawley rats	GD6-PND150; feed	3000, 10000, 3000 ppm	female	Pregnancy outcomes & maternal parameters F0 and F1 females	10000	No effects were observed on % pregenant F0 or F1 females or gestation length. In the two highest dose groups, dam (F0) body weights were significantly decreased during gestation (at GD21, 5% and 10% and body weight gains GD6-21: 11 and 25%) and in the 3000 group lactation (LD28, 10%). No clinical observations or effets on food conumsption were observed. [during gestation doses corresponded to approximately 205, 697, and 2644 mg/kg/day] In the two highest dose groups, pregnant F1 females showed lower gravid uterine weights (15% and 17%, respectively), fewer implants, and fewer live fetuses than control animals. Like for the F0 females, body weights in the two highest dose groups were also affecte inF1 females during lactation.
					Litter parameters (F1)	10000	No effects were observed on F0 litter size, PND1 pup weight, or sex ratio. Also, no effect on pup survival was observed. During lactation pup body weight decreased. On PND18, pup body weights were significantly decreased by 9-10% and 24% compared to controls in the two highest dose groups.
					Litter parameters (F2)	30000	In the high dose group, live litter size was reduced.
					AGD, F1 and F2		At PND1 there were no significant effects on AGD or AGD adjusted for bw observed, however,

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
							AGDs were generally between 5-8% shorter in exposed groups.
					Body weight F1 and F2 females	30000	In the 30000 female mean body weights were significantly decreased on PND91. [doses corresponded to approximately 287, 983, and 3593 mg/kg/day for PND28-91.
					Vaginal opening F1	30000	In the high dose group, day of vaginal opening was significantly later than in conrols (day 3 vs day 38), after adjusting for body weight at weaning this delay was mitigated to only 1 day.
					Estrous cyclicity	10000	Females in the 1000 and 30000 ppm groups displayed a higher probability of extended estrus and spent approximately 5% mre time in estrus than did the control grop. In the 10000 ppm group, the proestrus length was significantly decreased.
					Fertility F1 females		No effects were observed on the precoital interval and number of females that mated and number of pregnant females.
					Liver and kidney weight F1 females	3000	Increased relative liver weights and kidney weight were observed at necropsy at PND28 in all dose groups.
					Ovary and adrenal weight	10000	In the 10000 and 30000 ppm groups, females displayed lower absolute but not relative right and left ovarian weights. In the 30000 ppm group, also significantly decreased absolute adrenal gland weights were observed.
				male	AGD, F1 and F2		No significant effext observed.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
					Body weight F1 and F2	10000	In the highest dose groups mean body weights were significantly decreased on PND91.
					Nipple retention & testicular descend F1 and F2 males	30000	At PND13, no nipples were retained. No nipples were recorded in any of the male rats examined. In the high dose group, the day of testis descent was significantly before controls for F1, but not for F2 male rats.
					Balano-preputial separation F1 males	10000	In the two highest dose groups a delay was observed. When adjusted for body weight on day of weaning, these delays were no longer significant. In the 30000 ppm group, three males had not achieved BPS as of PND59, when checks of the marker finished. These males were from the same litter. None of them demonstrated evidence of mating or resultant evidence of pregnancy. At scheduled necropsy, two of the males had achieved BPS and the other had a hypospodia.
					Sperm parameters F1 males	30000	No effects on motile sperm, progressively motile sperm, or testis spermatid head concentrations were observed. In the 30000 group, males displayed lower cauda epididymal sperm counts.
					Reproductive organ weight F1 males	10000	In the 30000 ppm group, epididymis weight was decreased. In the two highest dose groups, testis weight was lower. These findings were not associated with histopathological changes or significant changes in reproductive performance. In the high dose group, absolute ventral prostate gland weights were decreased.

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
					Liver and kidney weights and histopahtology	3000	In the high dose group, kidney malformations were observed. In all dose groups, absolute and relative kidney weights were increased, and also relative liver weights.
Santamaria et al., 2020; Prenatal exposure, two subsequent pregnancies,	Female C57BL/6J and male BALB/c mice	• 7 days, • GD0-6, • dermal	50 mg/kg/day	female	Implantations		BP-3 treatment did not affect the number of implantations and abortions at either first or second pregnancies.
					placenta	50	Placental area, diameter and thickness, measured via ultrasound at GD10 and GD12, were comparable among groups. In the second pregnancy, but not in the first, placental weight was lower on GD14.
					Fetal parameters	50	At GD14, fetal weight form BP-3 treated mice was significantly reduced in the first pregnancy, no differences were observed in the second pregnancy. Male offspring bw was lower on PND4 and PND10-19. Femal offspring bw was lower at PND10 and 13 after first pregnancy, no differences in the second.
					Sex ratio	50	The percentage of females was higher (31% increase) in first and second pregnancies of mothers exposed to BP-3 than to controls.
Skórkowska et al., 2020; Prenatal and postnatal	Sprague Dawley rats (females only)	Entire gestation (22-23 days) + PND 43-56, **dermal**	100 mg/kg/day, Twice daily	female	Short-term and spatial memories	100	Effects on spatial memory, but not on short-term memory were observed.
					ER and PR mRNA and protein expression in brain	100	In frontal cortex, mRNA level of ERα, ERβ, and GPR-30 was significantly reduced. In the hippocampus, only ERβ expression was increased. At protein level, no effects were observed. However, in the hippocampus the PR

Reference; study design	Species	Exposure/route	Dose	Sex	Effect target	LOEL	Observed effect
							protein (cytosol, but not nuclear, fraction) was decreased compared to controls.
					AhR mRNA and protein expression in brain	100	Compared to the control, AhR mRNA level in the hippocampus was increased, whereas protein levels were decreased. No effects were observed in the frontal cortex.
					Protein expression of apoptotic markers in brain		No effects on protein expression of apoptotic markers was observed both in hippocampus and frontal cortex.
					Blood hormones	100	No effects were observed on 17β-estradiol, testosterone or prolactin concentrations, but decreased progesterone levels and increased free T3 and T4 levels were observed, whild TSH levels were decreased.
Pomierny et al., 2019; Gestational exposure	Sprague Dawley rats	GD0-22 and PND43-56; dermal	100 mg/kg/day	male	Behaviour, male offspring	100	No effects were observed on the discrimination index in the novel object recognition test and on short-term memory. However, rats exposed to BP-3 showed a significantly lower discrimination index in the novel location recognition test and thus weakened spatial memory.
					Brain gene and protein expression and transmitter levels in male offspring	100	The anti-oxidant capacity in the frontal cortex and hippocamus was reduced in BP-3 exposed rats, and lipid peroxidation and induced apoptosis were increased. The concentration of extracelluar glutamate in frontal cortex and hippocampus was increased in BP-3 exposed rats and the expression of glutamate transporters weas changed. No effects on body weight were observed.

Part IX

Substance monograph for **acetamiprid** and derivation of HBM guidance values for the general population

Technical reference

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Lead Beneficiary/ Responsible AE	UBA
Contributing Participants	Manca Ahačič (NIJZ), manca.ahacic@nijz.si Lucija Perharič (NIJZ), lucija.perharic@nijz.si Paul Scheepers (Radboudumc), paul.scheepers@radboudumc.nl
Responsible author(s)	Falko Partosch on behalf of UBA
Co-authors	Petra Apel (UBA), petra.apel@uba.de
Internal Reviewers[2]	WP4 co-leaders
External Reviewers[3]	Experts from, Belgian NH, UK NH Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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[1] PU = Public

PP = Restricted to other programme participants (including the Commission Services)

RE = Restricted to a group specified by the consortium (including the Commission Services)

CO = Confidential, only for members of the consortium (including the Commission Services)

[2] WP/task co-leader(s) from the same WP

[3] Independent Reviewers that should not be part of the task related to the AD/D or part of projects related to the Task

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Final version	14.03.2025	For approval by AD 4.3 reviewer (EAA)	Update of calculations now based on ADI of 5 µg/kg bw/d (Revision 8 of the Renewal Report by the Standing Committee on Plants, Animals, Food and Feed) and updated 24h bodyweight-related urine volumes (German HBM Commission). Further explanation of the selection of the toxicokinetic study for the derivation of the HBM-GVs. Deletion of information that is not relevant for the HBM-GV derivation. Information given on the approval and use as active substance in biocidal products.

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Abstract

Acetamiprid is a widely used chloropyridinyl neonicotinoid insecticide that is used against insects on numerous agricultural products. However, due to the wide range of applications, acetamiprid has raised concerns regarding its potential health effects. This is why it was included in the list of substances prioritized for HBM under PARC and why an HBM guidance value (HBM-GV) for the toxicological assessment of HBM results should also be derived.

A systematic literature search was conducted at Pubmed and Scopus on acetamiprid and its metabolites. Until April 2024, 1,355 publications were found, of which 140 were considered relevant. The most recent ones were further evaluated. In addition, existing authority reports were evaluated and the limit values derived from them were taken into consideration.

The most critical effects were identified in reproductive toxicity studies in animals.

An ADI of 25 µg/kg bw/d was proposed by EFSA in 2013 referring to developmental neurotoxicity. The animal study, which the ADI is based on is still regarded as the critical study after the literature search. But more recently, EFSA published a statement on the toxicological properties and maximum residue levels of acetamiprid and its metabolites where it was proposed to reduce both the ADI and ARfD values by a factor 5, i.e. from the current 25 to 5 µg/kg bw/d due to uncertainties in the evidence for developmental neurotoxicity properties (EFSA, 2024). According to Revision 8 of the Renewal Report the lower reference values have been finalised as part of the evaluation (SANTE/10502/2017 Rev 8). Also, the new MRLs are already published and will enter into force in August 2025 that reflect the lower ADI and ARfD, cf. para. 3 and 4 of the recitals in https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L_202500158.

N-Desmethyl-acetamiprid (DME-ACE) was found to be the biomarker of first choice, being a major and specific metabolite of acetamiprid. The urinary mass balance approach was used for this first-choice biomarker to derive HBM-GVs.

Based on the updated value (EFSA, 2024) and the updated values for the bodyweight-related urine volume of 30 mL/kg bw/d for men and 35 mL/kg bw/d for women (German HBM Commission, 2025, submitted), the HBM-GV for the major specific metabolite DME-ACE for children and men is 84 µg/L urine, for women 72 µg/L urine[49].

Key Words

HBM guidance values, HBM-GV, acetamiprid, N-desmethyl-acetamiprid

[49] By using the outdated 24h bodyweight-related urine volume for men and women of 0.02 L/(kg bw x d) for the calculation of the HBM-GV, the resulting HBM-$GV_{GenPop\ (adults)}$ would have been 127 µg/L urine.

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Authors and Acknowledgements

Authors

Falko Partosch on behalf of UBA

Petra Apel, UBA, Germany

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We would like to thank the experts who provided us feedback on this work during the consultation period. Comments were taken as far as possible into consideration for the finalisation of this report. An overview of the substantive comments and how they were dealt with can be found in the annex to this deliverable comprising various reports.

Background

Within the framework of PARC, health-based HBM guidance values (HBM-GVs) are established for priority substances as needed for the general population (HBM-GV_{GenPop}) and/or for workers (HBM-GV_{worker}) after their derivation approach underwent a consultation process by national experts nominated by PARC partners. The general methodological approach was published in 2020 (Apel et al., 2020).

In the current report, the derivation of HBM-GV_{GenPop}, both for children and adults, is presented and discussed for acetamiprid respectively its specific main metabolite N-Desmethyl-acetamiprid (DME-ACE) in urine.

Results

Based on the updated ADI value of 5 µg/kg bw/d (EFSA, 2024; SANTE/10502/2017 Rev 8) and the updated values for the 24h bodyweight-related urine volume of 30 mL/kg bw/d for men and 35 mL/kg bw/d for women (German HBM Commission, 2025, submitted), the HBM-GV for the major specific metabolite DME-ACE for children and men is 84 µg/L urine, for women 72 µg/L urine.

Abbreviations and Acronyms

6-CNA	6-Chloronicotinic acid
6-CNA-gly	6-Chloronicotinic acid, glycine conjugate
AAOEL	Acute Acceptable Operator Exposure Level
ACE	Acetamiprid
AChE	Acetylcholinesterase
AChR	Acetylcholine Receptors
ADI	Acceptable Daily Intake
AGD	Anogenital Distance
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AOEL	Acceptable Operator Exposure Level
ARfD	Acute Reference Dose
ASG	Atypical Symptomatic Group
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area Under the Curve
BBR	Berberine
BChE	Butyrylcholinesterase
BfR	Bundesinstitut für Risikobewertung
BMDL10	Benchmark Dose Lower confidence Limit (for 10% Benchmark Response)
BMI	Body Mass Index
CA	Chromosomal Aberration
cAMP	Cyclic Adenosine Monophosphate
CAS	Chemical Abstracts Service
CHD	Congenital Heart Diseases
CHO	Chinese Hamster vary
CKD	Chronic Kidney Disease
CLH	Harmonised Classification and Labelling
CLO	Clothianidin
Cmax	Maximum Measured Concentration
CSF	Cerebro-Spinal Fluid
DF	Detection Frequencies
DIN	Dinotefuran
DME-ACE	N-Desmethyl-acetamiprid
DNA	Deoxyribonucleic Acid

DNEL	Derived No Effect Level
DNT	Developmental NeuroToxicity
EC	European Commission
ECHA	European Chemicals Agency
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EPA	Environment Protection Agency
FGR	Foetal Growth Restriction
FLUP	Flupyradifurone
FMI	Fat Mass Index
FSH	Follicle-Stimulating Hormone
GD	Gestation Day
GI	Gastro Intestinal
GLP	Good Laboratory Practice
GPx	Glutathione Peroxidase
GSH	Glutathione
GTCS	Generalized Tonic Clonic Seizure
HBM	Human Biomonitoring
HBM-GV	Human Biomonitoring Guidance Values
HCD	Historical Control Data
HCT	Haematocrit
HD	High Dose
HDL-C	High-Density Lipoprotein Cholesterol
HGB	Haemoglobin
IARC	International Agency for Research on Cancer
IMI	Imidacloprid
IUPAC	International Union of Pure and Applied Chemistry
JECS	Japan Environment and Children's Study
Km	Michaelis constant
LD	Low Dose
LD50	median lethal dose
LH	Luteinizing Hormone
LMI	Lean Mass Index
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
LOQ	Limit of Quantification
LPO	Lipid Peroxidation

MD	Mid Dose
MDA	Malondialdehyde
MNCL	Mononuclear Cell Leukaemia
MRL	Maximum Residue Level
MWM	Morris Water Maze
NAC	N-Acetyl-L-cysteine
NEOs or NNIs	Neonicotinoids
NMDA	N-methyl-D-aspartate
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NSG	Non-Symptomatic Group
NSRL	No Significant Risk Level
OECD	Organisation for European Economic Co-operation
PBK	Physiologically Based Pharmacokinetic
PCNA	Proliferating-Cell Nuclear Antigen
PLT	Platelet count
PND	Postnatal Day
POD	Point Of Departure
PPAR	Peroxisome Proliferator-Activated Receptor
PU	Polyurethane
PXR	Pregnane X receptor
QSAR	Quantitative Structure Activity Relationships
RAC	Risk Assessment Committee
RBC	Red Blood Cells
ROS	Reactive Oxygen Species
SHE	Syrian Hamster Embryo
SOD	Superoxide Dismutase
SULF	Sulfoxaflor
TC	Total Cholesterol
TDI	Tolerable Daily Intake
TG	Test Guideline
TH	Thiamethoxam
THD	Thiacloprid
THI	Thiacloprid
THIAC	Thiacloprid
THIAM	Thiamethoxam
THM	Thiamethoxam

THX	Thiamethoxam
tmax	Time to maximum concentration
tRNA	Transfer Ribonucleic Acid
TRV	Toxicity Reference Value
TSG	Typical Symptomatic Group
Vmax	Maximum response amplitudes
WBC	White Blood Cells
WHO	World Health Organization

1 Introduction

Acetamiprid is a chloropyridinyl neonicotinoid insecticide that is used for the control of sucking-type insects on leafy vegetables, fruiting vegetables, cole crops, citrus fruits, pome fruits, grapes, cotton and ornamental plants and flowers[50] (El-Bialy et al., 2020) . It acts as a selective agonist of nicotinic acetylcholine receptors (nAChR) in the central nervous system of insects and is one of the most widely used neonicotinoids of the third generation, used for agricultural and domestic purposes against a large variety of insects. It is highly effective against pests on crops, fruits and leafy vegetables as well as against fleas infesting domestic livestock and pet animals like cats and dogs.

Neonicotinoid insecticides are applied through foliar sprays to above-ground plants, root drenches to the soil or trunk injections to trees. Acetamiprid among other has been found in fruit, vegetable, rice, honey and tea samples over the recent years according to (Cheng et al., 2020). Acetamiprid has also been reported to accumulate in plants and contaminate water which can pose a potential risk for human health (Arıcan et al., 2020; El-Bialy et al., 2020).

Acetamiprid was firstly approved in the EU as active substance for plant protection products by Commission Directive 2004/99/EC with the approval being renewed by Commission Implementing Regulation (EU) 2008/113 for further 15 years until 2033 (EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2022).

Furthermore, acetamiprid is approved until 31. January 2027 as an active substance for use in biocidal products of product-type 18, subject to the specifications and conditions set out in the Annex (http://data.europa.eu/eli/reg_impl/2018/1129/oj). Only one product, TECHNIVERT, is authorised for limited usage for termite control in France (national authorization).

[50] https://pubchem.ncbi.nlm.nih.gov/source/hsdb/7274#section=Manufacturing-Use-Information

2 Methodology

Relevant agency reports and risk assessments for acetamiprid were identified by general desk research.

For a systematic literature search of scientific publications, a search string was developed consisting of (A) a health part and (B) synonyms for acetamiprid and its major metabolites (Table IX.1). The full search term is shown in the Appendix.

Table IX.69: Parts of the search term used to search for scientific publications

Health part	health OR adverse OR toxic* OR neurotox* OR epidem* OR cohort OR genotox* OR genetic* OR carc* OR cancer OR reproduc* OR reprotox* OR developmental OR fertil* OR disease OR neurologic* OR metabol* OR adme OR pbpk OR pbtk OR excret* OR {in vivo} OR {in vitro} OR {in silico} OR endocrine OR {half-life} OR immun* OR moa OR {mode of action}
Synonyms for Acetamiprid and its major metabolites	Acetamiprid OR "IC-0" OR "6-Chloronicotinic acid" OR "IM-0" OR "IM-1-2" OR "IM-1-4" OR "1 6 chloropyridin 3 yl n methylmethanamine" OR "IM-1-5" OR "IM-2-1" [OR *additional synonyms of all substances*]

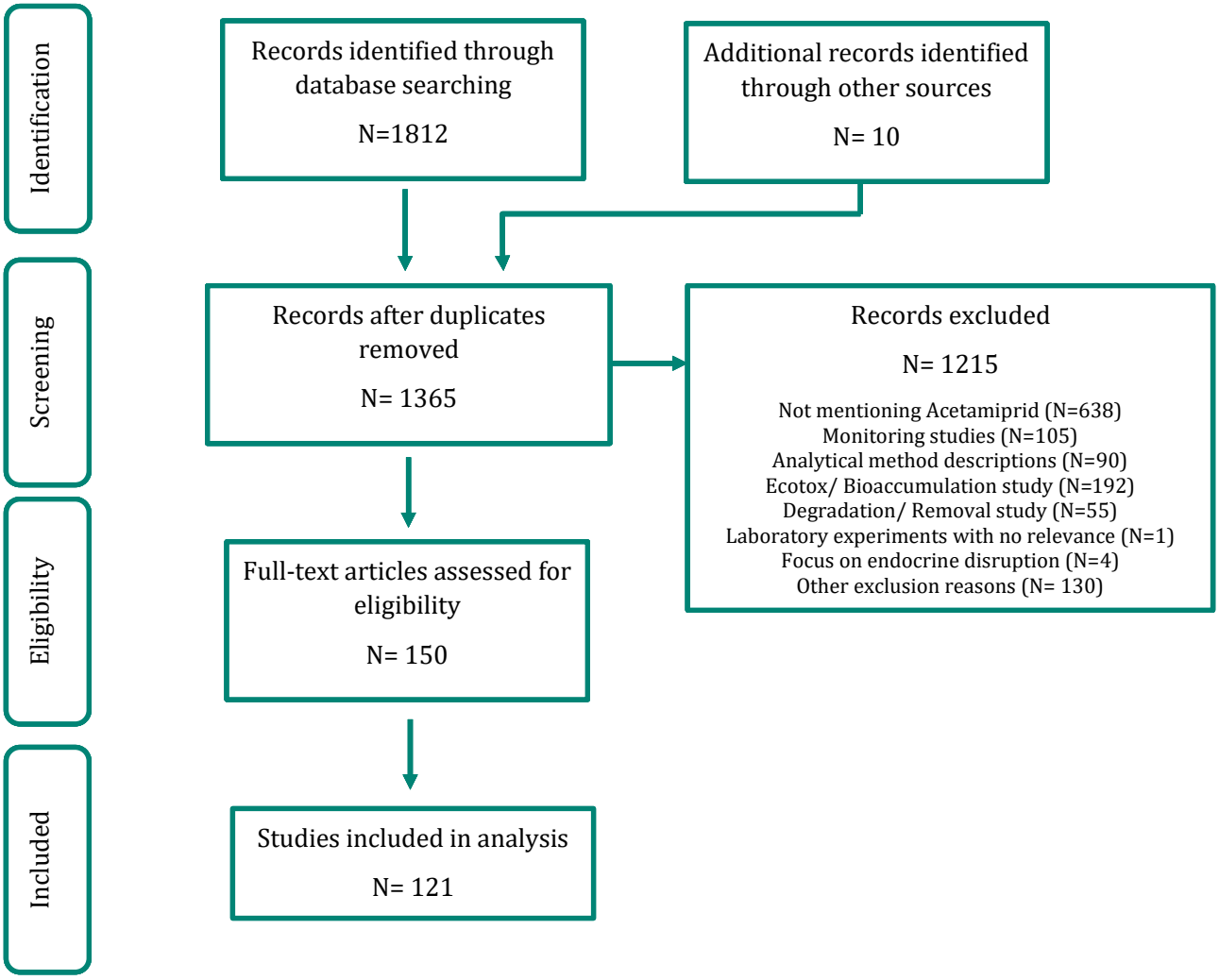
The search was carried out in SCOPUS and Pubmed and revealed a total number of 1355 publications that were published from January 2020 until April 2024. Further, 10 publications were identified through other sources. The 1365 publications were screened for relevance according to title and abstract using DistillerSR[51]. In this first step, 1215 publications were excluded. The remaining 150 publications were screened by full text and relevant information are discussed in the following chapters of this report.

The following exclusion criteria were applied:

- No information on acetamiprid or its metabolites
- Study type not of interest (e.g. analytical methods, removal, laboratory experiments)
- Studies with no information relevant for the deviation of human biomonitoring guidance values

[51] https://www.distillersr.com/products/distillersr-systematic-review-software

The following PRISMA diagram shows the process graphically.

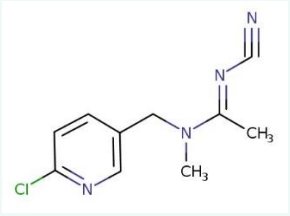


3 General characteristics

3.1 Physico-chemical properties

The information on physico-chemical properties of acetamiprid is based on the corresponding CLH report (CLH report, 2018) and the Hazardous Substance Databank[52].

Table IX.70: Substance characteristics and physicochemical properties of acetamiprid

Name	Acetamiprid
IUPAC names	(1E)-N-[(6-chloropyridin-3-yl)methyl]-N'-cyano-N-methylethanimidamide; (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine
EC no.	603-921-1
CAS no.	135410-20-7
Sum formula	$C_{10}H_{11}ClN_4$
Structural formula	
Appearance [1013 hPa; at 20°C]	Pure a.s. (99.9%): white fine powder, with no characteristic odour
Molecular weight [g/mol]	222.678
Relative density [g/cm³; at 20°C]	1.33
Melting point [°C; at 1013.25 hPa]	98.9
Boiling point [°C; at 1013 hPa]	Not relevant
Vapour pressure [at 50°C]	$1.73x10^{-7}$ Pa, Expected $<1x10^{-6}$ Pa at 25°C
Log P_{ow} [at 25°C]	0.8
Water solubility [g/L; at 25°C]	4.25 pH 5: 3.48 g/L at 25°C (>99%); pH 7: 2.95 g/L at 25°C (>99%); pH 9: 3.96 g/L at 25°C (>99%)
Dissociation constant [pK_a (= -log K_{10a})]	0.7
Classifications/ Hazard statements	Acute Tox. 3; H301, Repr. 2; H361d, Aquatic Acute 1; H400, Aquatic Chronic 1; H410

[52] https://pubchem.ncbi.nlm.nih.gov/source/hsdb/7274

3.2 Use of the substance

Acetamiprid is a pyridyl-methylamine, a neonicotinoid insecticide acting on insects by contact and ingestion, affecting the central nervous system. It is used in agriculture as an insecticide to control herbivorous (sucking and biting) insects and is applied as a foliar spray on crops (CLH report, 2018). It targets the nicotinic acetylcholine receptors in insects. Acetamiprid is a new-generation insecticide that is an alternative for controlling insect pests because of its lower toxicity to honeybees. Compared to other pesticides, acetamiprid is easily absorbed by plants and reacts swiftly at low concentrations. The intended use of acetamiprid is to control flies, cockroaches, ants, and other crawling and flying insects in residential and other buildings, in stables and animal transport utilities, as well as interfaces and external (non-garden) perimeter of buildings.

In 2013, acetamiprid, among others, was approved as active substance for use in plant protection products in the EU. Unlike other neonicotinoids, like imidacloprid, the use of acetamiprid was not restricted since EFSA established a low risk of the substance to bees. Therefore, its approval was renewed in the European Union until 28 February 2033 in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011[53].

Furthermore, acetamiprid is approved until 31. January 2027 as an active substance for use in biocidal products of product-type 18, subject to the specifications and conditions set out in the Annex (http://data.europa.eu/eli/reg_impl/2018/1129/oj). Only one product, TECHNIVERT, is authorised for limited usage for termite control in France (national authorization).

Acetamiprid has a harmonized classification according to annex VI of the CLP regulation (Regulation (EC) No 1272/2008) with Acute Tox. 3; H301 (toxic if swallowed), Repr. 2; H361d (suspected of damaging the unborn child), Aquatic Acute 1; H400 (very toxic to aquatic life) and Aquatic Chronic 1; H410 (very toxic to aquatic life with long lasting effects)[54].

3.3 Possible route of exposure

According to (Zhang and Lu, 2022) residents in both urban and rural areas are primarily exposed via dietary intake. In addition, contaminated water intake, soil/dust/pollen ingestion and inhalation are potential exposure routes for rural residents, especially children. According to the authors and the 2018 European Union report on pesticide residues in food, 0.08 – 0.35 % of food products in the European market contained acetamiprid, thiamethoxam, CLO and ACE above the maximum residue level (MRL), and 0.06 % contained acetamiprid exceeding the acute reference dose (ARfD), although EFSA considered it unlikely that the limited number of exceedances of the ARfD would pose concerns for consumer health (EFSA, 2020; Zhang and Lu, 2022). For insecticide operators, the primary route of exposure is probably dermal contact (Zhang and Lu, 2022).

There is extensive data on human exposure and biomonitoring in humans. In a review from 2022, data on human environmental exposure was evaluated. Most data were surveyed in China, for Europe only data from Greece was available for exposure to acetamiprid. However, it was remarkably, that urinary levels of neonicotinoids varied across countries and for acetamiprid they were mostly below LOD (Zhang and Lu, 2022). The highest urinary levels were found in adolescents from Southwest China with a value of 2.35 µg/g creatinine (Zhang and Lu, 2022) and in the Chinese general population with 0.014 µg/L urine (Zhang and Lu, 2022). For Europe, 118 Greek farm workers were examined where the concentration of acetamiprid was below the LOD of 2 ng/L as well (Zhang and Lu, 2022). According to the authors, there is an urgent need for further investigation of exposure and associated health risks. In addition, most of the studies explored in the review have not considered the metabolites, which makes it difficult to evaluate the data (Zhang and Lu, 2022).

[53] https://food.ec.europa.eu/plants/pesticides/approval-active-substances-safeners-and-synergists/renewal-approval/neonicotinoids_en
[54] https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/26276

4 Toxicological (toxicity) reference values proposed by European or relevant non-European institutions

The following Table IX.3 summarises available toxicological reference values.

Table IX.71: Available toxicological (toxicity) reference values.

Agency	Type of value	Value	Key study	Exposure route	Critical effect	POD	AFs	Source
JMPR, 2005	ADI	0-70 µg/kg bw/d	Anonymous 1999e: 2-year chronic carc. study Anonymous 1999d: 2-gen. reproductive study	Anonymous 1999e: Oral diet Anonymous 1999d: Oral diet	Anonymous 1999e: clinical signs + hepatocyte vacuolation at 17.5 mg/kg bw/d Anonymous 1999d: decreased parental body weight gain and feed consumption and hepatocyte vacuolation at 18.9 mg/kg bw/d	NOAEL = 7.1 mg/kg bw/d (Anonymous 1999e) supported by a NOAEL of 6.67 mg/kg bw/d (Anonymous 1999d)	100-fold combined uncertainty/adjustment	EFSA 2022 JMPR 2005
JMPR, 2005	ARfD	100 µg/kg bw	Anadón et al., 2020: Acute neurotoxicity study Anonymous 2008: Developmental 1-gen neurotoxicity study	Oral gavage	Anadón et al., 2020: Neurotoxicity, decreased locomotor activity and increased urination frequency Anonymous 2008: reduced bw gain in dams during the first 3 days of dosing (GD 6–9)	Anadón et al., 2020: NOAEL = 10 mg/kg bw based on acute neurotoxicity in rats supported by 10 mg/kg bw/d for reduced bw gain (Anonymous 2008)	100-fold	EFSA 2022 JMPR 2005

Agency	Type of value	Value	Key study	Exposure route	Critical effect	POD	AFs	Source
EFSA, 2013 ***European Commission 2018***	ADI, ARfD, AOEL, AAOEL	25 µg/kg bw/d, 25 µg/kg bw	Developmental neurotoxicity	Oral	Reduced auditory startle response in offspring	NOAEL = 2.5 mg/kg bw/d	100-fold combined uncertainty/adjustment	European Food Safety Authority (EFSA), 2016, 2022
EFSA, 2024	ADI, ARfD	5 µg/kg bw/d, 5 µg/kg bw	Developmental neurotoxicity	Oral	Reduced auditory startle response in offspring	NOAEL = 2.5 mg/kg bw/d	100-fold combined uncertainty/adjustment plus additional uncertainty factor of 5	SANTE/10502/2017 Rev 8, 13 December 2017, 24 September 2024)

5 Toxicokinetic and toxicodynamic profile

5.1 Findings in humans

Numerous epidemiological studies are available with exposure to acetamiprid. Most of them were carried out in Asian regions. However, in most cases, there is not only exposure to acetamiprid alone, but exposure to several neonicotinoids. Due to this and due to the numerous different investigation endpoints, there is no clear determination of the most predominant and common effects related to exposure to acetamiprid.

As no precise information on exposure is available in most epidemiological studies, the studies are not suitable to derive an HBM guidance value.

An overview of the studies can be found in Table IX.72 in the Appendix.

5.1.1 Human toxicokinetics and metabolism

Human volunteer studies describe that acetamiprid is rapidly metabolized by demethylation to N-desmethyl-acetamiprid (DME-ACE) after oral intake and that this is the primary specific metabolite which is suitable as a biomarker (

Table IX.73 of the Appendix). See Figure 1 below for general proposed metabolic pathway.

In most human toxicokinetic studies, the concentrations in plasma or urine are consistently higher for the metabolite DME-ACE than for the parent compound.

There are three relevant toxicokinetic studies in humans available, which have also been reviewed and approved by the relevant ethics committees in accordance with internationally recognised standards. In the first human toxicokinetic study, a micro dose of a mixture of deuterium-labelled neonicotinoids (5 µg each of acetamiprid-d6, clothianidin-d3, dinotefuran-d3, and imidacloprid-d4) was orally ingested by each of nine healthy adults without indication of gender. For a period of 4 days, 24 h pooled urine samples were collected from each individual. Most of the acetamiprid was metabolised to DME-ACE and unidentified metabolites. After 96 hours, the amounts of neonicotinoids excreted in urine were measured (no information on faeces). The recovery in urine of 5 µg deuterium-labelled acetamiprid was 0.13±0.17 µg (mean±SD), corresponding to 2.6±3.4 %, for the deuterium-labelled metabolite DME-ACE, the recovery rate was 1.53±0.78 µg, corresponding to 30.7±15.6 %. According to the authors, about half of the dose administered was metabolized into unidentified metabolites (Harada et al., 2016).

In the second study, by Wrobel et al. (Wrobel et al., 2022), the metabolism and excretion in urine of acetamiprid was investigated after a single oral dose at the EFSA ADI of 25 µg/kg bw/d (EFSA, 2013) which corresponded to a total dose of 2 mg in a single male volunteer. Post-dose urine samples were collected consecutively and completely up to 48 h. During the analysis, the parent compound and two putative metabolites, DME-ACE and 6-chloronicotinic acid-glycine (6-CNA-gly), were detected in the urine samples. However, the second metabolite 6-CNA-gly could not be confirmed using a second analytical method and moreover the metabolite is not specific to acetamiprid. Unchanged acetamiprid (ACE) was detected up to 24 h post-dose in urine. The metabolite DME-ACE appeared in the first urine sample after dosing (3.9 h) and remained at more or less constant levels up to 48 h post-dose (Wrobel et al., 2022).

In the third study, Wrobel et al. (Wrobel et al., 2023) performed a quantitative investigation of excretion kinetics. Urine samples from (Wrobel et al., 2022) were used, and two male volunteers (37 years, 98 kg; 48 years, 96 kg) were additionally administered individual single oral doses of acetamiprid of 2 mg among others (individual single oral doses of selected NNIs (ACE, CLO, FLUP, and IMI, all doses were administered with a time interval of at least 4 weeks to avoid interference). For ACE and DME-ACE the Cmax was 20 ± 7 and 520 ± 232 µg/g creatinine, respectively, two non-specific metabolites (6-chloronicotinic acid (6-CNA) and 6-CNA-gly) had Cmax values of 1.3 ± 1.1 and 142 ± 97 µg/g creatinine, respectively. Tmax for ACE and DME-ACE was 4.3 ± 4.0 and 8.1 ± 4.0 h, respectively, half-life values were 2.5 ± 1.2 and 19.1 ± 0.3 h, respectively. For ACE, the recovery rate was 0.48 ± 0.24 % after 24 h and 0.48 ± 0.25 % after 48 h. For the DME-ACE the recovery rates were 33 ± 6% and 54 ± 6% after 24 and 48 h, respectively (Wrobel et al., 2023).

Justification for the choice of the study to determine the urinary excretion fraction (Fue)

One can criticise that Wrobel et al. (2023) only collected urine for 48 h, whereas Harada et al. (2016) collected urine for 96 h, as it can be seen that the elimination of DME-ACE is not complete after 48 h (thus slightly overestimating potential NNI exposures). However, then it is astonishing that Harada et al. (2016) found lower Fue despite longer sampling time.

It is also unlikely that the differences could be due to the dosage. It would then be more likely that the Fue would be lower at higher doses (e.g. as a result of saturation of the enzymes involved in metabolism or the transport processes). Induction of metabolic enzymes is also not relevant with a single dose.

So, the discrepancy here seems to be the accuracy and correctness of the DME-ACE measurement. In addition to the low dosage (and the associated high demands on the detection sensitivity of the method), the fact that external calibration was used without an internal standard plays a role here[55]. This means that analyte losses and matrix effects could not be compensated for. In this respect, the content determination of DME-ACE by Harada et al. (2016) is subject to a significantly higher uncertainty than in Wrobel et al. (2023).

A low number of volunteers is very common in kinetic studies in humans for HBM purposes, and multiple studies have shown that important toxicokinetic parameters, such as $t_{1/2}$, tmax, and Fue's, are usually in good agreement between the volunteers. The observed interindividual difference in Fue (48h) between subjects is small for DME-ACE (range: 49-61 %).

Although background levels of DME-ACE were measured in Wrobel et al., these were insignificantly low: 'The use of ccorr in the case of DME-ACE did not change the Fue compared to uncorrected data, as post-dose concentrations were much higher than pre-dose concentrations (i.e. >1000-fold higher in t1).' For ACE itself, there was no measurable background exposure.

Conclusion: The background levels observed for DME-ACE in Wrobel et al. (2023) are irrelevant for the derivation of the Fue. Even if the shorter duration of urine collection in Wrobel et al. (2023) is a disadvantage, the lower Fue of Harada et al. (2016) is not very plausible for DME-ACE precisely because of the longer duration of urine collection. Overall, the Fue of Wrobel et al. (2023) is therefore more reliable.

As far as the PBPK model by Harada et al. (2016) is concerned, there is very good agreement between the measured recoveries and the modelled values for r from the one-compartment model for the parent substances. For DME-ACE, on the other hand, the agreement is not so good (two-compartment model), so that the PBPK modelling is no alternative.

5.2 Animal findings and in vitro studies

5.2.1 Animal toxicokinetics and metabolism

Acetamiprid radioactively labelled and orally dosed in rats was rapidly absorbed and distributed into organs and tissues via blood. A major part of radioactivity was excreted, mainly into urine through the kidney and to a lesser extent in faeces via bile. Sex differences in the metabolic profiles were not observed. When comparing the difference between the faecal and bile excretion rates, it can be seen that an enterohepatic circulation occurred during the elimination of acetamiprid. The radioactivity disappearance from the rat's body was rapid, and there was no indication of accumulation in any tissue. Determination of the metabolites showed that most of the acetamiprid (>90%) is metabolised, mostly to IC-0 (6-CNA, 6-Chloronicotinic acid) and IM-2-1 (DME-ACE, N-desmethyl-acetamiprid). The main metabolic pathway of acetamiprid in rats is the transformation to IM-2-1 (DME-ACE) by demethylation followed by further metabolisation (for chemical structures, please see Figure 1 Figure VI.3). According to (Arıcan et al., 2020), after oral administration the highest concentration is determined in the liver, kidney and thyroid glands.

[55] Further information on the Harada et al. study: Acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam were quantified using the internal standard method, using the deuterium-labeled analogue of each analyte as the internal standard for that analyte. The external standard method was used for the other target analytes, e.g. DME-ACE.

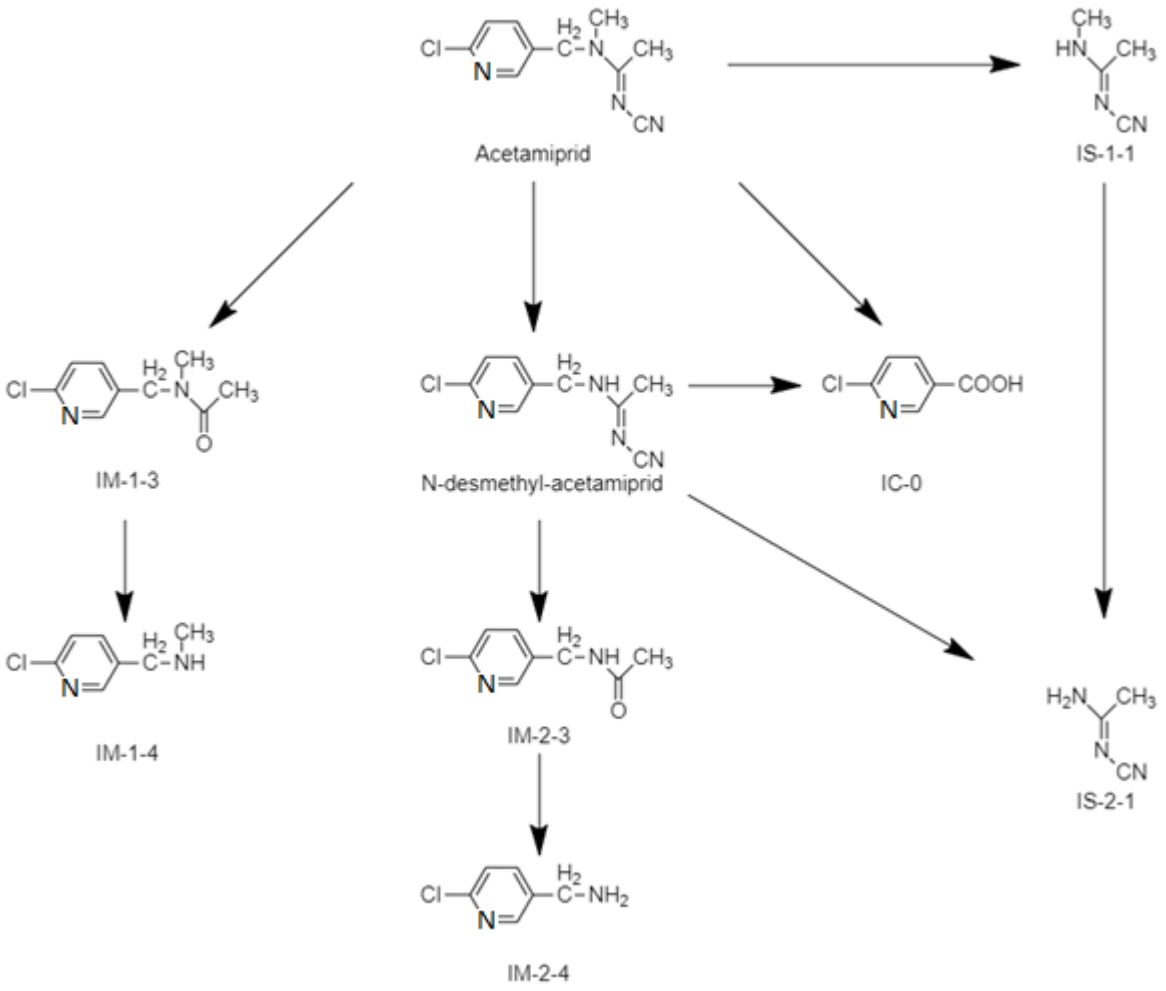


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Figure IX.9: Proposed metabolic transformation pathway for acetamiprid in rats. Acetamiprid (M=222.7 g/mol); N-desmethyl-acetamiprid (IM-2-1, M=208.7 g/mol) is the major metabolite (EC 2018[56]).

In a GLP compliant study, SD rats were orally exposed to 1 mg/kg bw/d [^{14}C]acetamiprid for 15 days. Group I-III (3/sex/group) were sacrificed 1, 10 or 96 h after dosing, groups IV and V (5/sex/group) 96 h after being exposed to unlabelled acetamiprid for 14 days following a single dose of radioactively labelled acetamiprid. In blood drawn from rats of group III 1 h post-dosing on days 1, 3, 7, 15, average concentrations in blood reached 0.477-0.747 and 0.465-0.698 µg/mL in males and females, respectively. Elimination half-lives (t½ (k)) for the male and female rats were 4.42 ± 1.10 hours and 5.56 ± 1.93 hours, respectively. Pharmacokinetic parameters for both sexes did not differ considerably and Tmax values indicated a rapid rate of absorption with a maximum blood concentration within 2-3 hours. Acetamiprid was rapidly absorbed (>90% within 1h) from the GI tract with most of it (53–65%) was excreted in the urine, excretion in urine and cage rinse combined amounted to 61-73%. Radioactivity, after administration of the last dose, was detected at the earliest sampling point (1 hour) in all the tissues (kidney, lung, pancreas, spleen, heart, brain, testes (male), ovary (female), skeletal muscles, inguinal fat (white), skin with hair, thyroid, bone, adrenal glands, gastrointestinal tract with contents) collected from each rat, being highest at 1 hour post-dosing in most tissues and declining rapidly thereafter. 10 hours post-dosing (group II), the residue levels were substantially lower. After 96 hours (group III) the residue levels were even lower. The highest radioactivity levels were observed in the GI tract, liver and kidney in both sexes at all sacrifice times, lowest concentrations were observed in bone and white fat (FAO and WHO, 2012).

[56] Acetamiprid Product type 18: Evaluation of active substances under Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products. Belgium 2018

In a further GLP compliant study, rats were intravenously or orally exposed to 1 or 50 mg/kg bw radioactively labelled acetamiprid. After 1 mg/kg bw, Tmax was 0.5-2 h and after 50 mg/kg bw, it was 3-7 h. The absorption rate after oral administration was >95% when comparing urinary excretion rate of oral to urinary excretion rate of i.v. exposure. After reaching Cmax in blood, levels decreased linearly and rapidly with half-lives of radioactivity being 5.84–7.11 h for group B and 8.07–15.03 for group D. Excretion rates in urine were high throughout the different groups with consistently >70% in urine. Total excretion rates were above 90% in all groups. Tissue concentrations were lower in bone and fat tissues than in blood, adrenal, thyroid, liver and kidney (FAO and WHO, 2012).

Further information on toxicokinetic studies is available in Table IX.75.

In summary, the available in vivo studies show that acetamiprid is widely distributed throughout the body after oral exposure with the majority of the radioactivity being excreted into the urine and to a lesser extent into faeces. After exposure and distribution, the substances disappear rapidly from the body with no tissues that are presumed to accumulate.

5.2.2 Acute toxicity with single administration

The acute toxicity of acetamiprid was investigated in several studies via the oral, dermal and inhalation route of exposure. There are 7 acute toxicity studies available via the oral route in rats, one in mice and one in birds with the lowest LD50 being 140-200 mg/kg bw and 146 mg/kg bw in rats, and LD50 being 194 mg/kg bw in mice and 129 mg/kg bw in birds. Acute dermal toxicity studies in rats show high LD50 values of greater than 2000 mg/kg bw. For the inhalation route of exposure studies in rats show LD 50 values of greater than 0.3 mg/L and greater than 1.15 mg/L. It was concluded by EFSA that acetamiprid is toxic by the oral route of administration but has low acute dermal and inhalation toxicity (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013).

In addition, there are several oral toxicity studies in rats with different metabolites of acetamiprid with LD50 values of 104 mg/kg bw to values greater than 5000 mg/kg bw.

Studies are summarised in Table IX.76 of the Appendix.

5.2.3 Neurotoxicity

There are 14 studies available dealing with neurotoxic effects of acetamiprid. The primary adverse effects observed include tremors, unsteadiness, and increased muscle tone, indicating the neurotoxic impact of acetamiprid on motor control and autonomic nervous functions. Studies on rats reported dilated pupils, increased urination, reduced body temperature, and altered locomotor activity, particularly at higher doses.

In an acute oral neurotoxicity study, increased urination frequency and reduced locomotor activity were observed at doses of 30 mg/kg bw and above. The NOAEL was 10 mg/kg bw, based on evidence of increased urination frequency in males and a statistically significant reduction of locomotor activity in males at 30 mg/kg bw (FAO and WHO, 2012).

A 13-week dietary neurotoxicity study in rats did not result in any changes considered indicative of neurotoxicity. The NOAEL was 200 ppm (equal to 14.8 mg/kg bw/d), based on lower body weights and feed consumption at 800 ppm (equal to 59.7 mg/kg bw/d) (FAO and WHO, 2012).

Long-term exposure (90 days) to one dose (3.14 mg/kg bw/d - LOAEL) altered respiratory potential and membranous permeability in brain mitochondria and lead to clear perturbation of neurotransmitter levels (Gasmi, 2020; Phogat et al., 2022). In another study, gliosis, necrosis and hyperaemia in brain tissue architecture have been seen after seven days of exposure to 5 mg/kg bw (Phogat et al., 2022).

A developmental neurotoxicity study in rats revealed the NOAEL to be 10 mg/kg bw/d, based on deficits in auditory startle response without neuropathology or changes in brain morphometry in F1 animals at 45 mg/kg bw/d (Gasmi, 2020) (FAO and WHO, 2012). The lowest NOAEL was established in a developmental 1-generation neurotoxicity study (Anonymous 2008 as cited in (RAC Opinion, 2020). A decreased pup acoustic startle response occurred in the mid and high dose group with 10 and 45 mg/kg bw/d, respectively, leading to a NOAEL of 2.5 mg/kg bw/d.

In vitro studies assessed neurotoxicity of acetamiprid across different cell types and concentrations. It has been found a significant impact of acetamiprid exposure on neurite outgrowth and changes in gene expression in rat PC12 Cell Line, indicating potential neurotoxic effects that can affect neural development and function (Christen et al., 2017; Kimura-Kuroda, 2016 as cited in NTP 2020). DNA damage was observed in cell line SH-SY5Y (Phogat et al., 2022). Another study shows alterations in Ca^{2+} influxes and neural excitation in rat neonatal cerebellar neurons (Kimura-Kuroda et al., 2012 as cited in NTP 2020) suggesting that exposure can lead to disruptions in neural signalling and excitability, which are critical for proper brain function.

Studies are summarized in Table IX.77 (*in vivo*) and Table IX.85 (*in vitro*) in the Appendix.

5.2.4 Subacute, sub-chronic and chronic toxicity

Acetamiprid has been investigated in a large number of studies with repeated exposure in mice, rats, rabbits and dogs with oral exposure via the diet or by gavage. Studies are summarised in Table IX.78 (Appendix). In almost all studies, liver and kidney effects were detected, which were reflected in increased organ weights and altered clinical chemistry parameters. In long-term studies, the observations were accompanied by histopathological changes.

5.2.4.1 Subacute exposure

Short-term studies of oral toxicity in mice, rats and dogs were conducted using acetamiprid. These studies are characterized by similar toxic responses, such as decreased feed consumption, decreased body weight, and histological alterations in liver and kidney tissue.

5.2.4.2 Sub-chronic exposure

In a 13-week study in mice, the no-observed-adverse-effect level (NOAEL) was 400 ppm (equal to 53.2 mg/kg bw/d), on the basis of a significant decrease in total cholesterol level in females at 800 ppm (equal to 106.1 mg/kg bw/d) (FAO and WHO, 2012). Tremor, decreased body weight gain, decreased feed consumption, decreased haemoglobin concentration, decreased serum total cholesterol and glucose levels, decreased urinary pH, increased liver to body weight ratio and centrilobular hypertrophy were observed at higher doses. In a 90-day study of oral toxicity in rats, the NOAEL was 200 ppm (equal to 12.4 mg/kg bw/d), on the basis of decreased body weight gain, decreased feed consumption and increased serum total cholesterol levels at 800 ppm (equal to 50.8 mg/kg bw/d) (FAO and WHO, 2012). In a 13-week study in rats, the no-observed-adverse-effect level (NOAEL) was 600 ppm (equal to 36.5 mg/kg bw/d), on the basis of a significant decrease in feed consumption and body weights (FAO and WHO, 2012). Decreased food intake, body and organ weight along with changes in hepatic and renal tissues were observed at intermediate and high dose in the 90-day study in rats with NOAEL ≤55 mg/kg bw/d (Phogat et al., 2022). In another study (13-week) the NOAEL of 12 mg/kg bw/d has been established based on the hepatotoxic as well as neurotoxic effects found in rats (Didenko et al., 2022). In a 60-day study of oral toxicity in rats, the NOAEL was 5 mg/kg bw/d based on dose dependent decrease in serum calcium and total protein and in serum oxidative/antioxidant biomarker levels (EL-Hak et al., 2022).

5.2.4.3 Chronic exposure

60 SD rats/sex/group were exposed for two years to 0, 160, 400, 1000 ppm in the diet, corresponding to 0, 7.1, 17.5, 46.4 mg/kg bw/d in males and 0, 8.8, 22.6, 60.0 mg/kg bw/d in females. Mean body weights and food consumption were significantly decreased at 400 and 1000 ppm. At 1000 ppm relative liver weights were increased, reaching significance in males only. In males from 400 ppm and in females at 1000 ppm, trace to mild hepatocellular hypertrophy was observed and a mild hypertrophy in males from 400 ppm. Trace to severe micro concretions in the renal papillae were observed in males at 1000 ppm. Therefore, the NOAEL in this study is 160 ppm, corresponding to 7.1 and 8.8 mg/kg bw/d for males and females, respectively (FAO and WHO, 2012).

In the second chronic study, 60 mice/sex/dose were exposed dietary over a period of 18 months to 0, 130, 400 or 1200 ppm acetamiprid, corresponding to 0, 20.3, 65.6, 186.3 mg/kg bw/d in males and 0, 25.2, 75.9, 214.6 mg/kg bw/d in females. Mean body weights and mean body weight gains were significantly reduced at 400 ppm in males with decreased food consumption in males at 1200 ppm. In females at 400 ppm, liver weights were increased. In males and females of the high dose group a statistically significant increase in hepatocellular hypertrophy was

observed at the interim sacrifice. At the terminal sacrifice, the incidence of amyloidosis was increased in several organs in high dose animals and in the adrenal cortex and kidney from 400 ppm. However, these findings were within historical control data and therefore not considered treatment related. The NOAEL in this study was 130 ppm, corresponding to 20.3 and 25.2 mg/kg bw/d in males and females, respectively (FAO and WHO, 2012).

5.2.5 Effects on skin and mucous membranes

Acetamiprid is described as not irritating to skin with a NOAEL of 1000 mg/kg bw/d and eyes by the JMPR which was further confirmed by medical surveillance of manufacturing plant personnel in Japan 1995 where no reports of any skin or eye irritation was reported (ECHA, 2013; FAO and WHO, 2012; United States Environmental Protection Agency, 2022). In addition, results from studies on skin sensitisation give no indication of sensitising potential which is also concluded by the EFSA (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013), for an overview see Table IX.11.

5.2.6 Genetic toxicity

The genotoxicity of acetamiprid has been investigated in a comprehensive range of *in vitro* and *in vivo* assays. The test system results are a negative Salmonella reverse mutation test with and without metabolic activation, a negative HGPRT gene mutation assay in Chinese hamster ovary (CHO) cells with and without metabolic activation, a negative unscheduled DNA synthesis in primary rat hepatocytes. There was a positive result in a chromosome aberration test in CHO cells with and without metabolic activation but only in combination with medium cytotoxic effects. Two comet assays were also positive with DNA damage in a dose dependent manner in combination with cytotoxicity. *In vivo* assay results were a negative micronucleus test in CD-1 mice, a negative chromosomal aberration test in bone marrow of SD rats and an *in vivo* unscheduled DNA synthesis test in SD rats also with negative results. Studies are summarised in

Table IX.80 of the Appendix.

The positive results in the chromosomal aberration test *in vitro* in CHO cells were only in combination with medium cytotoxic effects and according to the CLH report this was regarded not to be relevant since a negative in vivo mouse micronucleus test was available (CLH report, 2018). DNA damage was also observed in two comet assays in the studies of Annabi et al. (2019) and Şenyildiz et al. (2018) but only in combination with cytotoxic effects. In addition, further *in vitro* and *in vivo* tests had negative results.

RAC (2020) and (FAO and WHO, 2012) concluded that it is unlikely that acetamiprid is genotoxic and according to EFSA, acetamiprid was not found to be genotoxic (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013). However, the recently published *in vitro* comet assays by Senyildiz et al. (2018) and Annabi et al. (2019) as cited in a review by (Zuščíková et al., 2023) gave positive results in a dose dependent manner and it was concluded in the review that acetamiprid has genotoxic properties but this conclusion was solely based on both positive *in vitro* comet assays.

Since there is no OECD guideline for *in vitro* comet assays and the *in vivo* comet assay used for local genotoxicity is given more weight in assessments nowadays, it is still unlikely that acetamiprid is genotoxic, considering the negative in vivo results and the fact that positive results appeared only in combination with cytotoxicity.

5.2.7 Carcinogenicity

Carcinogenicity data for acetamiprid are available from two studies conducted in rats and mice via the oral route. Studies are summarised in

Table IX.81 in the Appendix.

In an 18-month GLP compliant study, 60 mice/sex/dose were exposed to dietary concentrations up to 1200 ppm, corresponding to 186.3 mg/kg bw/d in males and 214.6 mg/kg bw/d in females. Survival was not affected and there were no treatment related neoplastic effects observed in the study. According to RAC (2020) and (FAO and WHO, 2012) there was no evidence of an oncogenic effect.

In the second study, 60 SD rats/sex/dose were exposed to dietary concentrations up to 1000 ppm, corresponding to 46.4 mg/kg bw/d in males and 60 mg/kg bw/d in females for a period of two years. Survival was again not affected and there were no proliferative lesions in any treatment group of either sex that were considered to be definitively test substance related. Mammary gland hyperplasia was observed in 49% of the subjects in the top dose group, but this was within the historical control data, which spans from 0% to 58.6%. A significant increasing trend in mammary gland adenocarcinoma was observed, with incidences of 10, 11, 24 and 29 at 0, 160, 400 and 1000 ppm, respectively. The increase of 28.3% in the top dose was within the historical control range of 13.3% to 28.6%, according to (FAO and WHO, 2012). However, there was no significant difference to the control by pairwise comparison. According to RAC, this tumour is a common finding in SD rats. There was no known mode of action and the substance was not shown to be genotoxic (RAC Opinion, 2020).

Since the tumours were not clearly treatment related and confined to a single species with no such observations in mice, RAC considered the evidence for carcinogenic potential to be insufficient. The same conclusion that acetamiprid was not oncogenic in rats and mice was drawn by (FAO and WHO, 2012).

5.2.8 Reproductive toxicity

5.2.8.1 Effects on fertility

There are numerous studies available for acetamiprid with data on fertility effects. An overview on existing studies dealing with effects on fertility in animals can be found in Table IX.82 in the Appendix.

Based on the information available, the most predominant and common effects of acetamiprid exposure on fertility across various studies in different species (primarily rats and mice) can be summarized as follows:

Decreased testosterone levels, which have been reported in multiple studies analysing serum testosterone levels. Several studies observed a decrease in sperm count, motility, and viability, suggesting that acetamiprid has detrimental effects on sperm parameters.

However, these studies were not conducted according to any guideline and no such effects were observed in the most relevant key study, which is a two-generation study in rats according to OECD 416 where SD rats were exposed via food to 0, 100, 280, 800 ppm (0, 6.5/7.5, 17.9/21.0, 51.0/63.3 mg/kg bw/d for F0/F1 males, 0, 7.6/8.4, 21.7/23.8, 60.1/72.6 mg/kg bw/d for F0/F1 females during premating, 0, 6.8/6.6, 18.5/18.5, 50.9/55.2 mg/kg bw/d for F0/F1 females during gestation and 0, 13.2/13.7, 37.5/40.3, 108.1/105.5 mg/kg bw/d for F0/F1 females during lactation) at least 10 weeks prior to mating until weaning of F2. There were no treatment related deaths and no compound-related histopathological effects in F0 parents, but there was a decrease in relative kidney weights. In F1 parents, two females of the 280-ppm group and five females of the 800-ppm group experienced total litter deaths but no deaths of adults. Body weight and food consumption were decreased in the 800-ppm group. There were no compound related histopathological changes and no effects on reproductive organs or function. No change of anogenital distance was recorded for F1 pups but preputial separation and vaginal opening was significantly increased in correlation with decreased body weight. In F2 pups, viability and weaning indices were significantly affected in high dose (HD) animals. Pup mortality greatly increased from PND 0-21, and although body weight was slightly decreased, post-natal growth was strongly decreased. The mean age for reaching eye opening in high dose pups was significantly increased, correlating to decreased body weight.

It was concluded by (FAO and WHO, 2012) that no effects on reproductive capacity was observed up to the highest dose of 800 ppm. This conclusion was confirmed by ECHA (RAC Opinion, 2020) and EFSA (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013).

5.2.8.2 Developmental toxicology

The developmental toxic effects of acetamiprid have been investigated in a large number of studies. Studies are summarised in Table IX.83 of the Appendix.

The most predominant and common effects across the data provided are related to alterations in body weight, food consumption, offspring growth, and neurodevelopmental issues associated with acetamiprid exposure. Here is a summary of the most common findings:

A recurring effect observed in different species, including rats and rabbits, was a decrease in body weight and food consumption at higher doses of acetamiprid. This effect tended to be treatment-related and usually manifested at the higher dose levels.

Acetamiprid is classified as Repr 2, H361d (RAC Opinion, 2020). Studies showed developmental toxicity, which included decreased offspring body weights in utero and postnatally, delayed sexual maturation, and increased pup mortality. There were instances of total litter loss in high-dose groups and anomalies in foetal development, such as skeletal variations and decreased neurogenesis. There were no foetal anomalies on visceral findings despite a single high dose foetus with microphthalmia (abnormally small eyes). No external or skeletal findings were observed despite some sporadic non-significant differences in the type and incidence of skeletal anomalies in treatment groups, which lacked dose dependency.

Some studies in mice suggested that acetamiprid exposure could lead to neurodevelopmental toxic effects, microglial activation, and alterations in neurobehavioral responses (e.g., altered acoustic startle response, impaired spatial learning and memory).

In high-dose groups, there was a clear impact on offspring viability and weaning indices, with significant pup mortality and decreased postnatal growth.

The most important studies are described in the following. There are two GLP and OECD 414 guideline compliant studies in rats and rabbits where no teratogenicity or fetotoxicity was observed up to the highest doses of 50 and 30 mg/kg bw/d tested. (FAO and WHO, 2012).

In contrast, in a 2-generation study according to OECD 414 and in compliance with GLP, SD rats were exposed to 0, 100, 280, 800 ppm (0, 6.5/7.5, 17.9/21.0, 51.0/63.3 mg/kg bw/d for F0/F1 males, 0, 7.6/8.4, 21.7/23.8, 60.1/72.6 mg/kg bw/d for F0/F1 females during premating, 0, 6.8/6.6, 18.5/18.5, 50.9/55.2 mg/kg bw/d for F0/F1 females during gestation and 0, 13.2/13.7, 37.5/40.3, 108.1/105.5 mg/kg bw/d for F0/F1 females during lactation). There was a significant reduction in pup body weights in the top dose group for both sexes with a 7% decrease in utero and a more pronounced 11-24% reduction postnatally, affecting both F1 and F2 pups similarly. While the viability of F1 pups was not affected, the viability and weaning indices were significantly affected in high dose F2 pups with greatly increased pup mortality. In addition, there was a significant decrease in in-utero growth, post-natal growth and body weight during lactation in these pups. The NOAEL of this study is indicated by 280 ppm (FAO and WHO, 2012).

In a developmental 1-generation neurotoxicity study conducted according to EPA guideline 870.6300, SD rats were exposed to 0, 2.5, 10, 45 mg/kg bw/d via gavage from GD 6-21. There were signs of maternal toxicity in the top dose group with a single mortality due to dystocia (difficult or abnormal labour), decreased body weight gain and food consumption. Pup post-natal survival was decreased with total litter loss in three dams. A significantly decreased pup acoustic startle response with maximum response amplitudes (Vmax) and average response amplitudes (Vave) were observed in HD males when compared to the control group values on PND 20 and 60. A similar decrease was observed at 10 mg/kg bw/d without maternal toxicity but this effect was not statistically significant. Therefore, the NOAEL for developmental neurotoxicity was 2.5 mg/kg bw/d (FAO and WHO, 2012). This effect was also considered critical by EFSA (EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2022; European Food Safety Authority (EFSA), 2016).

In an evaluation of the RAC of ECHA, the effects listed led to the classification of acetamiprid as Repr 2; H361d (RAC Opinion, 2020).

5.2.9 Immunotoxicity

There are only few studies available dealing with immunotoxicity of acetamiprid. An overview on studies focussing on effects on the immune system can be found in Table IX.84 of the Appendix. Studies on immune functions in mice (highest dose tested was 157 mg/kg bw/d) and rats (highest dose tested was 67.7 mg/kg bw/d) indicated no specific effect on immune function as assessed by the measurement of antigen-specific T cell–dependent antibody formation (FAO and WHO, 2012).

6 Derivation of human biomonitoring guidance values

The purpose of human biomonitoring (HBM) studies is to identify and quantify chemicals of concern or their metabolites in human biological material with a subsequent toxicological interpretation of the measurements to determine if (further) chemical management measures or regulation are needed (Apel et al., 2020). Existing data on acetamiprid allows for the derivation of HBM guidance values (HBM-GVs) for the general population that correspond to internal exposure levels at which there is no appreciable risk and which can therefore be used as an assessment tool.

6.1 Choice of the critical effect and POD

According to (Apel et al., 2020), there are three options to derive the HBM-GV. The first option is to choose a POD according to the critical effect from human data, the second option is to use a defined external TRV, proposed by a European or relevant non-European institution and the third option is a POD based on a critical effect observed in experimental animal studies or human studies (which are sometimes available for occupational exposure settings). Since no adequate human data is available, the second and third options were examined (for an overview on TRVs see Table IX.3).

The choice of a critical effect should be based on prolonged exposure. There are two carcinogenicity studies in rats and mice with 24- and 18-months exposure, respectively. Tumours occurred only in rats and there were no tumours that could clearly be attributed to the treatment. However, non-neoplastic lesions occurred with clinical signs and hepatocyte vacuolation at 17.5 mg/kg bw/d leading to a NOAEL of 7.1 mg/kg bw/d.

The lowest NOAEL established, was in a developmental 1-generation neurotoxicity study (Anonymous 2008 as cited in (RAC Opinion, 2020)). A decreased pup acoustic startle response occurred in the mid and high dose group with 10 and 45 mg/kg bw/d, respectively, leading to a NOAEL of 2.5 mg/kg bw/d.

In 2013, EFSA established an ADI of 0.025 mg/kg bw/d due to the significantly decreased pup acoustic startle response in the developmental neurotoxicity study with a NOAEL of 2.5 mg/kg bw/d (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013; FAO and WHO, 2012). An uncertainty factor of 100 was applied to derive the aforementioned ADI of 0.025 mg/kg bw/d.

The same study was used to derive the acute reference dose (ARfD). This is due to the fact that effects in DNT studies cannot be clearly assigned to long term or short term effects and the observed developmental toxicity is relevant for long-term exposure. (https://echa.europa.eu/documents/10162/2324906/biocides_guidance_human_health_ra_iii_part_bc_en.pdf/30d53d7d-9723-7db4-357a-ca68739f5094?t=1512979002065, page 177).

More recently, EFSA published a statement on the toxicological properties and maximum residue levels of acetamiprid and its metabolites where it was proposed to reduce both the ADI and ARfD values by a factor 5, i.e. from the current 0.025 to 0.005 mg/kg bw/d due to uncertainties in the evidence for developmental neurotoxicity properties (EFSA, 2024). According to Revision 8 of the Renewal Report, the lower reference values have been finalised as part of the evaluation (SANTE/10502/2017 Rev 8). Also, the new MRLs are already published and will enter into force in August 2025 that reflect the lower ADI and ARfD, cf. para. 3 and 4 of the recitals in https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L_202500158.

6.2 Derivation of HBM-GVs

If no more differentiated PBPK models are available, an HBM-GV can be derived from a TRV as described in (Apel et al., 2020) by using the urinary mass balance approach knowing the rate of excretion of the active substance or its specific metabolite(s), the molecular weight of the parent substance and its metabolite(s) and the urinary flow rate related to the body mass.

The general formula for deriving an HBM-GV for one main metabolite is:

$$HBM - GV = TRV \times \frac{\left(\frac{MW(Metabolite) \times Fue(Metabolite)}{MW(Substance)}\right)}{Daily\ urinary\ flow\ rate}$$

For acetamiprid, DME-ACE was concluded to be a suitable biomarker for human biomonitoring (see section 5.1.1). Wrobel et al. (2023b) established a specific and robust multi-method to analyse 7 neonicotinoids and neonicotinoid-like compounds (NNIs) together with 9 specific metabolites with a limit of quantitation for acetamiprid of 0.06 µg/L and for DME-ACE of 0.05 µg/L. Urine samples are thereby enzymatically hydrolysed with β-glucuronidase and analysed using liquid chromatography-electrospray tandem mass spectrometry coupled to online-SPE (online-SPE-LC-ESI-MS-MS). Inter-day and intra-day imprecision was ≤6% for all analytes. The mean relative recovery was in the range between 88 and 112%.

In chapter 5.1.1 it is furthermore justified that the kinetics study by Wrobel et al. (2023) should be used to determine the Fue for the HBM-GV derivation. The Fue from this study is: 0.54 (54 %) after 48 h.

The molecular weight of the parent substance acetamiprid is 222.7 g/mol and its major metabolite DME-ACE is 208.7 g/mol.

According to a current data-based evaluation by the German HBM Commission (2025, submitted), the urine volume of adults per kg bodyweight and day has increased over time, so that the previously used standard value of 0.02 L/kg bw/day for adults needs to be updated. Thus, the following 24h bodyweight-proportional urine volumes per day enter the calculation: 0.03 L/kg bw/day for children and men and 0.035 L/kg bw/day for women.

When considering the ADI of 0.005 mg/kg bw/d proposed by EFSA 2024, which is the agreed reference value of the renewal revision report of acetamiprid and endorsed by the Standing Committee on Plants, Animals, Food and Feed in 2024, this results in the following HBM-GV for children and men:

$$HBM - GV = \frac{0.005\frac{mg}{kg}bw}{d} \times \frac{\left(\frac{208.7\frac{g}{mol} \times 0.54}{222.7\frac{g}{mol}}\right)}{\frac{0.030\frac{L}{kg}bw}{d}} = \mathbf{0.084}\frac{mg}{L}\ \boldsymbol{or}\ \mathbf{84}\ \mu\boldsymbol{g/L}$$

The HBM-GV for the major specific metabolite DME-ACE for children and men is thus: 84 µg/L urine; for women, the analogue value is 72 µg/L urine.

The derived HBM-GVs represent the concentration of DME-ACE in human urine up to which – for single substance evaluation – a risk for adverse health effects is not anticipated.

When assessing HBM results, it must be taken into account that exposure to mixtures of substances is usually present, so that the additional use of a MAF (mixture assessment factor/mixture allocation factor) or another suitable methodology should be considered for further assessments. At least structurally related substances or substances that display the same effect targets should be considered together for the next review and refinement of the HBM-GV.

7 Level of confidence attributed to the derived HBM-GVs

As mentioned in the HBM4EU concept document to derive HBM-GVs, it is suggested to attribute a level of confidence (low, medium or high) to each calculated HBM-GV. The level of confidence should reflect the uncertainties identified during the elaboration of the value and could constitute a good incentive to later revise values with an estimated better level of confidence.

The data used for calculation of urinary excretion fraction was derived from a study with three healthy male adult volunteers, who received a single oral acetamiprid dose of 2 mg (Wrobel et al., 2023). The observed interindividual difference of Fue between the volunteers for the main metabolite DME-ACE was below a factor of 2 which is within biological tolerance. Nevertheless, as toxicokinetics varies depending e.g. on the age, gender or ethnicity of the test person, uncertainty remains in the transfer of the Fue to all population groups.

However, since the ADI used already includes default safety factors to cover toxicokinetic intra- and inter-species variability, it can be concluded that the variability assumed is already covered to some extent but acetamiprid specificities were not taken into account due to lack of data. Therefore, the level of confidence regarding the Fue is **medium**.

The ADI is based on a one-generation developmental neurotoxicity study in rats according to EPA 870.6300 and in compliance with GLP (Nemec, 2003 as cited in (FAO and WHO, 2012)) with a NOAEL of 2.5 mg/kg bw/d for reduced auditory startle response in F1 males. The same study was identified as the key study in the performed literature search for this project. Hence, the TRV chosen is considered suitable. However, the key study for the derivation of the ADI is based on animal data. The best case for deriving HBM-GVs would have been availability of human data. More recently, EFSA published a statement on the toxicological properties and maximum residue levels of acetamiprid and its metabolites where it was proposed to reduce both the ADI and ARfD values by a factor 5, i.e. from 0.025 to 0.005 mg/kg bw/d due to uncertainties in the evidence for developmental neurotoxicity properties (EFSA, 2024). According to Revision 8 of the Renewal Report the lower reference values have been finalised as part of the evaluation (SANTE/10502/2017 Rev 8). Also, the new MRLs are already published and will enter into force in August 2025 that reflect the lower ADI and ARfD, cf. para. 3 and 4 of the recitals in https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L_202500158. Nevertheless, due to the uncertainties indicated, the level of confidence is considered to be **low/medium**.

The overall level of confidence is considered to be medium.

It should be noted, though, that the levels of confidence suggested in this document are based on expert judgement regarding the accuracy of the data and the process of calculation used to derive the HBM guidance values, rather than being established in accordance with a recognized and accepted methodology.

8 Factsheet reporting the HBM-GV_{GenPop} derivation

Acetamiprid (ACE)	**Target population:** **General population**	
Parameter	**Value / descriptor**	**Comments**
HBM-GV_{GenPop} and Status		
HBM-GV_{GenPop}	**Based on EFSA, 2024** The HBM-GV for the major specific metabolite DME-ACE for children and men is 84 µg/L urine, for women it is 72 µg/L urine.	
Level of confidence	Medium	
HBM-GV status	Agreed	
HBM-GV year of issue	2025	
General Information		
CLP-INDEX-Nr.	608-032-00-2	
EC-Nr.	603-921-1	
CAS-Nr.	135410-20-7	
Harmonised CLP classification	- Acute Tox. 3 – H301 - Repr. 2; H361d - Aquatic Acute 1 - H400 - Aquatic Chronic 1 - H410	- toxic if swallowed - suspected of damaging the unborn child - very toxic to aquatic life - very toxic to aquatic life with long lasting effects
Molar mass	222.678 g/mol	
Biomarker(s)		
Identification	N-desmethyl-acetamiprid (DME-ACE) in urine	
Molar mass of biomarker(s)	208.7 g/mol	
Half-life of selected biomarker(s)	19 hours	Wrobel et al., 2023
Derivation method, starting point and assessment factors		
Type of derivation method selected	Translation of ADI value of 0.005 mg/kg bw/d and ARfD value of 0.005 mg/kg bw into urinary specific metabolite DME-ACE concentration	by using the urinary mass balance approach (Apel et al., 2020) ADI and ARfD values as agreed by the renewal report of acetamiprid (SANTE/10502/2017 Rev 8) which considered the additional factor of 5 due to uncertainties in the evidence for developmental neurotoxicity properties (EFSA, 2024)

Acetamiprid (ACE)	**Target population:** **General population**	
Parameter	**Value / descriptor**	**Comments**
Key study	FAO and WHO, 2012 Developmental 1-gen. neurotoxicity study on pregnant female Crl:CD(SD) IGS BR rats (25/dose group)	
Exposure route of study	Oral (in feed)	0, 2.5, 10, 45 mg/kg bw/d
Critical endpoint and POD	Reduced auditory startle response in offspring NOAEL of 2.5 mg/kg bw/d	
Extrapolations and AFs	Global AF of 100 and additionally 5 for ARfD and ADI	EFSA, 2024, SANTE/10502/2017 Rev 8
Calculation of HBM-GV$_{GenPop}$		
Derivation of HBM-GV	From a TRV by using the urinary mass balance approach (Apel et al., 2020)	
Rounded value of HBM-GV$_{GenPop}$	The HBM-GV for the major specific metabolite DME-ACE for children and men is 84 µg/L urine, for women 72 µg/L urine.	

9 Appendix

9.1 Search Terms

Scopus

TITLE-ABS-KEY (acetamiprid OR {(E)-N1-(6-chloro-3-pyridyl)methyl-N-cyano-N-methylacetamide} OR ic-0 OR {6-Chloronicotinic acid} OR im-0 OR {6-chloro-3-pyridyl)methanol} OR ib-1-1 OR {N-(6-chloro-3-pyridyl)methylacetamide} OR im-2-3 OR {(E)-N-(6-chloro-3-pyridyl)methyl-N'-cyanoacetamidine} OR im-2-1 OR {N-(6-chloro-3-pyridyl)methyl-N-methylacetamidine} OR im-1-5 OR {1-(6-chloropyridin-3-yl)-N-methylmethanamine} OR im-1-4 OR {N-(6-chloro-3-pyridyl)methyl-N-methylacetamide} OR im-1-3 OR {(E)-N-carbamoyl-N-((6-chloropyridin-3-yl)methyl)-N-methylacetimidamide} OR im-1-2 OR {(E)-N-(6-chloro-3-pyridyl)methyl-N'-cyanoacetamidine} OR im-2-2 OR {(E)-N'-carbamoyl-N-((6-chloropyridin-3-yl)methyl)acetamidin} OR {N2-cyano-N1-methyl-N1-(3-oxo-2-2-azabicyclohex-5-en-6-yl)methyl-acetamidine} OR is-1-1 OR {(1E)-N'-Cyano-N-methylethanimidamide} OR is-2-1 OR {N-Cyanoethanimidamide}) AND TITLE-ABS-KEY (health OR adverse OR {mode of action} OR toxic* OR neurotox* OR epidem* OR cohort OR genotox* OR genetic* OR carc* OR cancer OR reproduc* OR reprotox* OR developmental OR fertil* OR disease OR neurologic* OR metabol* OR adme OR pbpk OR pbtk OR excret* OR {in vivo} OR {in vitro} OR {in silico} OR endocrine OR {half-life} OR immun* OR moa OR {mode of action}))

Pubmed

(("Acetamiprid"[Title/Abstract] OR ("IC-0"[Title/Abstract] OR "6-Chloronicotinic acid"[Title/Abstract]) OR "IM-0"[Title/Abstract] OR "IM-1-2"[Title/Abstract] OR ("IM-1-4"[Title/Abstract] OR "1 6 chloropyridin 3 yl n methylmethanamine"[Title/Abstract]) OR "IM-1-5"[Title/Abstract] OR "IM-2-1"[Title/Abstract]) AND ("health"[Title/Abstract] OR "adverse"[Title/Abstract] OR "toxic*"[Title/Abstract] OR "neurotox*"[Title/Abstract] OR "expos*"[Title/Abstract] OR "epidem*"[Title/Abstract] OR "cohort"[Title/Abstract] OR "genotox*"[Title/Abstract] OR "genetic*"[Title/Abstract] OR "carc*"[Title/Abstract] OR "cancer"[Title/Abstract] OR "reproduc*"[Title/Abstract] OR "reprotox*"[Title/Abstract] OR "developmental"[Title/Abstract] OR "fertil*"[Title/Abstract] OR "disease"[Title/Abstract] OR "neurologic*"[Title/Abstract] OR "metabol*"[Title/Abstract] OR "ADME"[Title/Abstract] OR "PBPK"[Title/Abstract] OR "PBTK"[Title/Abstract] OR "excret*"[Title/Abstract] OR "in vivo"[Title/Abstract] OR "in vitro"[Title/Abstract] OR "in silico"[Title/Abstract] OR "endocrine"[Title/Abstract] OR "half-life"[Title/Abstract] OR "immun*"[Title/Abstract] OR "MOA"[Title/Abstract] OR "mode of action"[Title/Abstract])) AND (2020:2024[pdat])

9.2 Findings in humans

Table IX.72: Overview of epidemiological studies evaluating neonicotinoid exposures and effects

Reference	Cited in	Findings
Marfo et al. 2015	(EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2022), NTP 2020	• Multiple insecticides including acetamiprid measured in spot urine samples collected from patients (4-87 years old) having participated in a clinical study in Japan over a period of three years (exposure timing unknown) • Patients lived close to agricultural fields • Categorised in three groups: typical symptomatic group (TSG, n=19), atypical symptomatic group (ASG, n=16), non-symptomatic group (NSG, n=50) • Typical symptoms defined as two objective symptoms (recent memory loss and finger tremor), and more than five of six subjective symptoms (headache, general fatigue, palpitation/chest pain, abdominal pain, muscle pain/weakness/spasm and cough) • DME-ACE and six other neonicotinoids were quantified in urine by LC-MS/MS • Frequency of detection of DME-ACE was highest in the TSG group (47.4%), followed by ASG (12.5%) and NSG (6.0%) with maximum concentrations of 6, 4.4, 2.2 mg/L, respectively • Detection of DME-ACE was associated with prevalence of symptoms, leading the authors to conclude that urinary DME-ACE could be used as a biomarker for environmental exposure • According to EFSA, the assessment of this study had several uncertainties and limitations, as most of the symptoms were non-specific and may be related to clinical conditions other than exposure to neonicotinoids + symptoms specific of stimulation of nicotinic acetylcholine receptors in the autonomic nervous system (e.g. tachycardia, hypertension, diaphoresis, and mydriasis) were not considered • Since DME-ACE was not detected in 52.6% of TSG and in 87.5% of ASG it is likely that the observed symptoms are not specifically linked to the exposure • No causality can be inferred from detection of DME-ACE in urine und presence of symptoms • Covariates like age, gender, food and tea intake were not included as potential predictors in the logistic regression analysis although available • Reduced sample size and use of a unique spot urine sample limit drawing robust conclusions • The maximum DME-ACE concentration was lower than in other studies
Takashiba 1997	(FAO and WHO, 2012)	• Medical surveillance of manufacturing plant personal in Japan during production from April 1995 until April 1997 • Helmet, safety goggles, safety shoes, filter respirator and rubber gloves as protective measures • Health examination once a year on all employees in the plant including physical examination, haematology (haemoglobin (g/dl), haematocrit (%), erythrocytes and white blood cells), urinalysis

Reference	Cited in	Findings
		(protein (mg/dl), glucose (mg/dl) and urobilinogen (mg/dl)), blood chemistry (ASAT, ALAT, AP, GGT, total cholesterol) • No significant changes in parameters since production had started
Todani et al. 2008	(FAO and WHO, 2012)	• Clinical case of acute acetamiprid poisoning with a 79-year old man who had ingested acetamiprid and received medical attention 2 hours after ingestion. • On arrival, he had consciousness disturbance (GCS-8), hypotension, nausea, vomiting and hyperglycaemia, but he had neither constricted pupils nor mucous super secretions, which are characteristic of organophosphate poisoning. • Gastric lavage was performed, activated charcoal and laxative were administered • Paroxysmal atrial fibrillation persisted until 11 hours after ingestion • Symptoms improved next day; he was discharged from the hospital without complication • Blood concentration of acetamiprid on arrival, approximately 2 hours after ingestion, was 21.1 µg/mL
Imamura et al. 2010	(FAO and WHO, 2012)	• Two cases of acute poisoning with an insecticide formulation containing acetamiprid for suicidal purposes • Both cases experienced severe nausea and vomiting, muscle weakness, hypothermia, convulsions and clinical manifestations, including tachycardia, hypotension, electrocardiogram changes, hypoxia and thirst in the case with the higher serum concentration of acetamiprid • Symptoms were partially similar to those of acute organophosphate intoxication • Supportive treatments for a variety of symptoms were sufficient for recovery • Both individuals were discharged without any complications 2 days after ingestion
Ichikawa et al. 2019	(EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2022)	• Developing an analytical method for seven neonicotinoids and the metabolite DME-ACE in human urine using LC–ESI/MS/MS • 65 very low birth weight (VLBW, 500–1500 g) infants of gestational age 23–32 weeks admitted to the neonatal intensive care unit of a Japanese Hospital over 2 years • Urine samples were collected in the first 2 days and at day 14 after birth • DME-ACE detected in 14 urine samples collected at birth (24.6%, median level 0.048 ppb) and in 7 samples from PND 14 (11.9%, median level 0.09 ppb) • Urinary DME-ACE detection rate and level was significantly higher in infants with shorter gestational age (small for gestational age (SGA) • DME-ACE levels showed no correlation with infant physique indexes (length, height, and head circumference SD scores) • Study was excluded by EFSA due to uncertainties and limitations like urine samples being collected at the same time as the outcome assessment which precludes any causal interference and missing information of in utero exposure

Reference	Cited in	Findings
		• According to EFSA, an error in the statistical analysis allowed false conclusions to be drawn; a correct analysis would have allowed the conclusion that the exposure took place postnatally • The sample size was relatively low • Some of the exclusion criteria are not comprehensible for Ramboll, as an earlier urine measurement would have been impossible and the urine content of PND 1 allows conclusions to be drawn about exposure in utero
(Ospina et al., 2019)		• To assess human exposure to neonicotinoids, 3038 US participants of the general population from 2015-2016 were examined • 49.1 had been exposed to neonicotinoids • Urinary concentrations of six neonicotinoid biomarkers were quantified with the major metabolite being DME-ACE (35%), the 95th percentile concentration was 1.29 µg/L • <0.5% of the detection frequencies was the parent substance acetamiprid • For people who fasted <8 h, regardless of race/ethnicity and sex, 3–5 year old children and 6-11 year old children were more likely to have DME-ACE concentrations above the 95th percentile than adolescents • Asians were more likely than non-Asians to have concentrations above the 95th percentile of DME-ACE, same goes for samples collected during the summer • The detection of the metabolite was more frequently and at much higher concentrations than the parent compound, which suggests that the metabolites may be suitable biomarkers to assess background exposures according to the authors.
(H. Wang et al., 2020)		• Human exposure to neonicotinoids in 309 Chinese school children aged 7-11 years from 2019 • Eight neonicotinoids and four metabolites were determined in 81.3 % of first morning urine samples, among them acetamiprid and its metabolite DME-ACE • DME-ACE was more likely to be detected in children who were older, physically active, or obese and in children who drank more tap water • Acetamiprid was found in the urine samples of three children (1%), its metabolite in 181 children (62.6%) • DME-ACE had the maximum 99th percentile of concentration (88.16 µg/g creatinine) and the maximum concentration (243.71 µg/g) of all neonicotinoids tested • An estimation of daily exposure was performed with the formula DED = Cn × Mc/(Cc × Mb × P × 1000) (DED: daily exposure dose, µg/kg/day; Cn: neonicotinoid concentration in urine, ng/mL; Cc: creatinine concentration in urine, mg/mL; Mc: daily output of creatinine in urine, mg/day; Mb: body weight, kg; P: neonicotinoid excretion proportion in urine as unchanged or metabolite forms) • A linear regression equation derived from 454 healthy children aged 3–18 years was used to predict Mc: common log-transformed Md = 0.0102 × H-0.6854 (Md: creatinine concentration in urine, mmol/day; H: body height, cm) (Mc = 113.1 × Md) • The excretion proportion of acetamiprid was 0.586 (based on DME-ACE)

Reference	Cited in	Findings
(Y. Chen et al., 2023)		• Cross-sectional study of cancer and non-cancer (case/control) cohort participants in a Chinese population; 201 subjects, 81 cancer patients, 120 non-cancer participants (female + males of age 20 - >80) • Quantification of the residual concentrations of eight individual NEOs in human bile and quantitatively link the hepatoxic outcomes to internal exposures of NEOs, and their associations with hepatotoxicity risks. • Approximately 3–5 mL of bile samples from each participant has been collected during the endoscopic retrograde cholangiopancreatography • Due to low detection frequency, residual ACE concentrations were not considered when comparing different groups. The average relative contribution of NIT of all compounds in bile samples was approximately 78%, followed by DIN (8.6%), IMID (3.6%), CLO (2.8%), and ACE (1.2%) and THI (0.98%)
(J. Chen et al., 2023)		• An individual who consumed the acetamiprid and pyridaben mixture for suicidal purposes, and subsequently developed central nervous system depression, hyperlactacidemia, and metabolic acid poisoning, which thus required clinical management with hospitalization; A 74-year-old woman was transported to hospital after ingesting a combination of 30 mL of 5% acetamiprid and 5% pyridaben . • The patient displayed nausea and vomiting, followed by confusion. An arterial blood gas analysis revealed metabolic acidosis and hyperlactacidemia. The patient was carefully monitored for vital signs and treated with gastric lavage, purgation, and proton pump inhibitors to reduce gastric acid (added: production), blood volume, and electrolyte resuscitation • Potassium and calcium replacement was performed for hypokalemia and hypocalcemia
(Gulen et al., 2022)		• Case report - A 57-year-old male patient consumed 50g/250mL of an insecticide formulation containing acetamiprid for suicidal purposes • Ambulance team performed cardiopulmonary resuscitation on patient who had a cardiac arrest. His heart beat returned after a 15-min cardiopulmonary resuscitation, and afterward, he had tachycardia, hypotension, respiratory failure, high gap metabolic acidosis with a high lactate, hypokalaemia, hypocalcaemia, mydriasis, and coma. The patient underwent supportive treatment for a variety of symptoms. However, he died despite all supportive treatment.
(Elangovan et al., 2022)		• Case report - A 45-year-old man was referred to our hospital emergency department with alleged history of ingestion of pesticide, which contained a combination of acetamiprid 4% (w/w) and fipronil 4%. He had a generalized tonic clonic seizure (GTCS), while being transported to hospital. He was intubated for airway protection and initiated on mechanical ventilation. He was commenced on midazolam for his seizure management. Gastric lavage was done, and activated charcoal was administered. • He made an uneventful recovery and was discharged home after 3 days of hospital stay.
(Pirasath et al., 2021)		• Acute poisoning with an acetamiprid in a middle-aged farmer for suicidal purposes.

Reference	Cited in	Findings
		• He presented with severe nausea, vomiting, and altered level of consciousness. He had electrocardiogram changes, hypoxia, and lactic acidosis. With intensive care management including ventilatory support, inotropic therapy along with intraarterial blood pressure monitoring, correction of acidosis, and administration of electrolytes, he made good clinical recovery. • He was discharged without any further complications 6 days after ingestion of acetamiprid.
(Pravinson et al., 2021)		• A case of acetamiprid poisoning in a 27-year-old man with a history of accidental intake of neonicotinoid poisoning about 50mL (commercial name is "MIYAKO ACETAMIPRID"). • At emergency services, he presented with vomiting, shortness of breath, and dizziness, developed severe lactic acidosis, myocardial suppression, hyperglycaemia, and intestinal obstruction. He received supportive management including gastric lavage and activated charcoal.
(Zhao et al., 2023)		• Study investigated the relationship between NNIs and their metabolites in urine with serum lipid profiles in US adults. • Participants from National Health and Nutrition Examination Survey (NHANES) 2015–2016 were included with 1192 individuals aged over 20 years with urinary NNIs levels, serum lipid parameter levels and potential confounders • Serum lipids profiles, such as total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B (Apo-B), were assessed • Significant increase in TC levels among participants with detectable DME-ACE compared to those with undetectable levels • DME-ACE had higher detection rates among non-drinkers, individuals with a college degree or higher, those with physical activity level ≥ 600 MET min/week • Participants with hypertension and diabetes had a lower detection rate for DME-ACE • Participants aged ≥ 46 years old with detectable DME-ACE had a higher HDL-C level compared to those aged 20–45 years old • BMI might modify the association between detectable DME-ACE and serum LDL-C and Apo-B level but the association is weak.
(Hua Zhang et al., 2022b)		• Investigation of 200 serum samples from an elderly population in China • DME-ACE was the most abundant NEO in the serum samples with the highest median concentration in the serum sample from populations with osteoporosis (OP) (5.99 ng/mL) and non-OP populations (2.02 ng/mL). • Median serum concentrations of target analytes ranged from 0.04 (ACE) to 5.99 (ACE-dm) ng/mL in population with osteoporosis and from 0.01 (THI) to 2.02 (ACE-dm) ng/mL in population without osteoporosis • The associations between NEOs and their metabolites and OP were significant. • According to the authors, the concentrations of NEOs and their metabolites are associated with the increased odds of OP prevalence.

Reference	Cited in	Findings
(Hua Zhang et al., 2022a)		• Association of exposure to neonicotinoid insecticides and their characteristic metabolites with human liver cancer in blood samples collected from healthy donors (n = 100; females vs. males: 45 vs. 55; age: 22–91 years) and liver cancer patients (n = 274; females vs. males: 118 vs. 156; age: 11–88 years) in one Chinese hospital • The detection rate of acetamiprid was 61% in the healthy group, vs. 80% in liver cancer patients with mean values of 0.93 vs. 1.39 ng/mL, respectively, for ACE-DM the detection rate was 93% vs. 91% and the mean values were 1.11 vs. 1.61 ng/mL, respectively. • However, the combined blood concentrations were considered for the association with human liver cancer and the median blood concentrations of NEOs and their metabolites ranged within 0.19–1.28 ng/mL for the healthy population and 0.20–2.03 ng/mL for the liver cancer population. • Significant positive correlations between NEO exposure and liver cancer were found, which revealed that NEOs concentrations were associated with increased odds of liver cancer prevalence for ACE the odds ratio was 0.42 (adjusted 0.28) and for ACE-DM the OR was 3.45 (adjusted 2.55).
(Zhang et al., 2021)		• Association of neonicotinoid insecticides and their metabolites in paired saliva and periodontal blood samples in human from South China with oxidative stress markers were measured in 188 paired samples. • A positive relationship was found between the concentrations of NEOs and their metabolites in saliva and periodontal blood and oxidative stress biomarkers of DNA damage.
(Q. Zhang et al., 2023b)		• Occurrence, distribution and potential risk to infants of neonicotinoids in breast milk were described in a case study from China. • Breastmilk samples were collected from Hangzhou Medical College from mothers within 2 months of delivery with overall 137 breast milk samples. • At least one neonicotinoid was detected in >94 % of the breast milk samples, with a detection frequency of 30.7% for acetamiprid. • The study is limited as there is lacking information about the corresponding metabolites which is crucial for acetamiprid which is rapidly metabolized.
(Yue et al., 2022)		• In a cross-sectional study, urinary neonicotinoid concentrations and pubertal development was investigated in Chinese adolescents. • 774 urine samples from 439 boys (median age: 13.7 years) and 335 girls (median age: 13.7 years) were collected for determination of ten neonicotinoids. • Surprisingly, the detection rate of ACE was 100% in the urine of boys and girls with a geometric mean value of 2.18 µg/g creatinine. • For DME-ACE, the detection rate was 99.4 % (99.3% in boys, 99.7% in girls) with geometric mean values of 18.17 µg/g creatinine for boys and girls • The influence on puberty was only assessed using all neonicotinoids; individual evaluations for acetamiprid are therefore missing

Reference	Cited in	Findings
(Godbole et al., 2022)		• Study investigated the association between detectable concentrations of parent neonicotinoids (imidacloprid, acetamiprid, clothianidin) and neonicotinoid metabolites (5-hydroxy-imidacloprid, DME-ACE) with adiposity measures (fat mass index (FMI), lean mass index (LMI), waist circumference, body fat percentage, and BMI) among US adults, and whether sex modifies the associations for metabolites. • Participants from NHANES 2015–2016 were included with 1675 examined participants (adults over 19 years) who had at least one reported urinary neonicotinoid measured along with at least one reported measure of adiposity and BMI. • The odds of being overweight or obese with detectable neonicotinoids were estimated using logistic regression. • Detectable concentrations of acetamiprid were inversely associated with adiposity - a decrease in FMI, LMI, body fat percentage, waist circumference and BMI among adults.
(Gu et al., 2023)		• Study investigated the association between the urinary neonicotinoid and the relative mtDNAcn (RmtDNAcn) of oral epithelial cells collected in a cross-sectional study including 35 postgraduates (25 male and 10 female) with repeated measurements over 6 weeks. • Imidacloprid and acetamiprid were the top two neonicotinoids that were frequently detected (54.3–74.3% for imidacloprid and 48.6–74.3% for acetamiprid). The mean concentration 0.52 μg/L (acetamiprid) after adjusting by SG during the whole six weeks
(Lu et al., 2023)		• Study aimed to assess urinary levels of NEOs/metabolites and their associations with children's adiposity measures, and to further investigate the potential role of oxidative stress. A total of nine indicators of adiposity were measured. • 380 children who participated in the 7th year's follow-up of the Laizhou Wan Birth Cohort in northern China. • Six NEOs [imidacloprid (IMI, 99.7%), clothianidin (CLO, 98.9%), dinotefuran (DIN, 97.6%), thiame-thoxam (THM, 95.5%), acetamiprid (ACE, 82.9%), thiacloprid (THD, 77.6%)] and two metabolites [N-desmethyl-acetamiprid (DME-ACE, 100.0%), 6-chloronicotinic acid (6-CINA, 97.9%)] exhibited high detection rates. • Statistically significant nonlinear associations were found in the relationship of ACE only with height z-score and weight z-score.
(Mendy and Pinney, 2022)		• Study aimed to assess the association of urinary neonicotinoids with serum testosterone in humans. • The data were analysed on 2014 male and female participants (NHANES conducted between 2015 and 2016) aged 6 or older. • Analysis of individual neonicotinoids was performed on 5-hydroxy-imidacloprid and DME-ACE; imidacloprid, thiacloprid, acetamiprid, and clothianidin were not studied as individual compounds due to their low urinary detection ranging from 0.7% to 7.9% • In linear regression adjusted for age and potential confounders, serum total testosterone was 37.78% lower with 10-fold increase in urinary total neonicotinoids, 20.81% lower with10-fold increase in urinary 5-hydroxy-imidacloprid) and 25.01% lower with 10-fold increase in urinary DME-ACE among males. Serum free androgen index (FAI) was also decreased with higher urinary DME-ACE. In

Reference	Cited in	Findings
		females, serum total testosterone was 32.91% lower with 10-fold increase in urinary total neonicotinoids, 21.32% lower with 10-fold increase in urinary 5-hydroxy-imidacloprid and 15.42% lower with urinary detection of 5-hydroxy-imidacloprid. FAI was likewise reduced with higher urinary levels of 5-hydroxy-imidacloprid and DME-ACE.
(Nishihama et al., 2023)		• The study aimed to examine the association between NEO exposure and child development up to 4 years of age using data of the Japan Environment and Children's Study (JECS). • JECS dataset included data from pregnancy to 4 years of age (n = 104,059 foetal records). Maternal urine samples and questionnaires data were collected twice during pregnancy (the first trimester: < 22 weeks of gestation [early] and the second or third trimester: ≥ 23 weeks of gestation [mid/late]). • The Japanese translation of the Ages and Stages Questionnaires, third edition (J-ASQ-3), was adopted to evaluate childhood development from 6 months to 4 years of age. The developmental delay has been scored in five domains: communication, gross motor skills, fine motor skills, problem solving, and personal-social characteristics. A total of 8538 participants were included in statistical analyses. • Median urinary DME-ACE, CLO, DIN and THX concentrations were 0.34, 0.14, 0.22 and 0.05 ng/mL, respectively, in samples collected during gestational weeks < 23, and 0.28, 0.12, 0.18 and 0.04 ng/mL, respectively, in those collected during gestational weeks ≥ 23. • No statistically significant association between maternal urinary DME-ACE, CLO, DIN and THX concentrations during pregnancy and the J-ASQ-3 results up to 4 years of age.
(Pan et al., 2023)		• The study aimed to quantify the single and combined associations of maternal exposure to NEOs and foetal growth restriction (FGR). • Nested case-control study based on the Guangxi Zhuang Birth Cohort, China (2015-2018) with 387 FGR cases and 1096 without FGR controls included. • Ten NEOs were measured from the maternal blood samples in the first trimester. • Models were adjusted for other NEOs chemicals and included sampling seasons, maternal age, birthplace, occupation, pre-pregnancy BMI, passive smoking, regular physical activity, parity, pregnancy complications, and infant sex. • For acetamiprid, an odds ratio of 1.31 (95% CI: 1.07, 1.59) was established for the association with the risk of FGR, for the metabolite DME-ACE, the OR was less than 1 at 0.86 (95% CI: 0.76, 0.98). • However, even if above 1, an OR of 1.31 is rather low and should not be overestimated.
(Qu et al., 2024)		• The study assessed the associations between NEOs and congenital heart diseases (CHDs). • Nested case-control study was conducted within an ongoing prospective birth cohort study and enrolled 141 CHD singletons and their 282 individually matched controls. Six "parent" NEOs and three NEO metabolites were measured in maternal serum collected at an average gestational age of 16 weeks.

Reference	Cited in	Findings
		• DME-ACE and imidacloprid (IMI) were the most frequently detected NEOs, found in 100% and 20% of maternal sera, respectively. • No statistically significant association between maternal urinary DME-ACE and CHDs.
(Sun et al., 2023)		• Cross-sectional study • 14 NEOs and their metabolites in urine samples of 500 individuals (236 and 264 with and without dyslipidaemia, respectively) randomly selected from the baseline of the Yinchuan community-dwelling elderly cohort (Ningxia, China). • The positive associations and dose-dependent relationships of thiacloprid, imidacloprid-olefin, and imidacloprid-equivalent total with dyslipidaemia were validated using restricted cubic spline analysis. Mixture models revealed a positive association between the NEOs mixture and dyslipidaemia risk, with urine desnitro-imidacloprid ranked as the top contributor. • THI, DME-ACE, and IMI-olefin may have positive non-linear relationships with the risk of dyslipidaemia
(Suwannarin et al., 2021)		• Cross-sectional study with 143 male farmworkers aged 18–40 years in Fang district, Chiang Mai province, northern Thailand • Urinary NEOs, their metabolites (NEO/m) and serum steroid hormones were measured using liquid chromatography–tandem mass spectrometry. Among • NEO/m, the GM concentration of IMI was highest (17.4 ng/mL), followed by DME-ACE (15.8 ng/mL), THX (9.1 ng/mL), CLO (7.4 ng/mL) and Of-IMI (5.1 ng/mL). • DME-ACE levels were positively associated with the androstenedione level
(Tang et al., 2024)		• Study aimed to analyse the concentrations of eight p-NEOs and five specific m-NEOs in the first urine collected from 92 newborns, both preterm and full-term, in southern China during 2020 and 2021. • NEOs and their metabolites were identified in 91 urine samples, with over 93% of samples containing a mixture of these compounds. • DME-ACE with detection frequency 87% and median of 0.291 ng/mL in the urine. No statistically significant association with preterm births were identified for DME-ACE.
(Vuong et al., 2022)		• Cross-sectional study examined associations between neonicotinoids with insulin and glucose homeostasis parameters among 1381 non-diabetic adults in NHNES (2015–2016). Urinary concentrations of acetamiprid, clothianidin, imidacloprid, N-desmethyl-acetamiprid, and 5-hydroxy-imidacloprid were quantified. Fasting plasma glucose, insulin, and haemoglobin A1c (HbA1c) were assessed. • Detection frequency for DME-ACE was 32.8%. • The only effect observed for DME-ACE was sexually dimorphic associations between DME-ACE and glucose; While sex-stratified findings did not reach statistical significance, males with detectable DME-ACE had higher levels of glucose compared to the lower glucose levels that were observed in females.
(A. Wang et al., 2022)		• Cross-sectional study investigating associations of neonicotinoid insecticide metabolites with semen quality.

Reference	Cited in	Findings
		• The concentrations of neonicotinoid insecticides and some of their metabolites (collectively defined as mNNIs) in seminal plasma samples collected from men (n = 191) who visited a fertility clinic in Shijiazhuang, North China from 2018-2019. • DME-ACE, detection frequency: 98.4% at median concentrations of 0.052 ng/mL. • No associations were found for DME-ACE.
(Wu et al., 2024)		• Study aimed to examine the association between urinary neonicotinoid concentrations and obesity measures among Chinese adolescent: 524 urine samples from 300 boys (11.3–16.1 years) and 224 girls (12.1–15.8 years) • DME-ACE concentration was associated with an increase in waist-to-height ratio and waist-to-hip ratio.
(Yang et al., 2024)		• Study aimed to examine the association between urinary neonicotinoid concentrations and obesity measures (Body mass index (BMI) and waist circumference (WC)) among Chinese children: 442 children in Shanghai, East China. • Children in the high urinary concentration of acetamiprid and its metabolite had a low BMI z-score.

Table IX.73: Overview of toxicokinetics, metabolism and biomonitoring studies in humans

Reference	Cited in	Findings
Li et al. 2020c	(Zhang and Lu, 2022)	- Urinary levels of neonicotinoids varied across countries with ACE mostly being below LOD
Zhou et al. 2021	(Zhang and Lu, 2022)	- Highest urinary value was established in adolescents from Southwest China with a value of 2.35 µg/g creatinine
Song et al. 2020	(Zhang and Lu, 2022)	- Also, high values in Chinese general population with 0.014 ng/mL urine
Li and Kannan 2020	(Zhang and Lu, 2022)	- In 118 Greek Farm workers the concentration of acetamiprid was below LOD
(Harada et al., 2016)		- A single micro dose of a mixture of deuterium-labelled neonicotinoids (5 µg each of acetamiprid-d6, clothianidin-d3, dinotefuran-d3, and imidacloprid-d4) was orally ingested by each of nine healthy adults, - 24 h pooled urine samples were collected for 4 consecutive days after dosing. - Excretion kinetics were modelled using one- and two-compartment models, then validated in a non-deuterium-labelled neonicotinoid micro dose study involving 12 healthy adults ingesting a single oral dose of a mixture of 2 µg each with 24h pooled urine samples collected on the day before and after the micro dose exposure with a spot urine sample being collected every 24 h until 168 h after ingestion for each participant. - Most of the acetamiprid was metabolized to desmethyl-acetamiprid but was eliminated more slowly than the other compounds used - After 96 h, the recovery in urine of 5 µg deuterium-labelled acetamiprid was 2.6±3.4 %, for deuterium-labelled Desmethyl-acetamiprid 30.7±15.6 %

Reference	Cited in	Findings
		- About half of the acetamiprid dose was metabolized into unidentified metabolites - Urinary excretion time courses for the 2 µg unlabelled neonicotinoid bolus test showed that the mean concentration of desmethyl-acetamiprid had increased after one day - Desmethyl-acetamiprid was considered as a suitable biological monitoring marker
(Wrobel et al., 2022)		- Human metabolism and metabolite excretion in urine of acetamiprid (ACE), clothianidin (CLO), flupyradifurone (FLUP), imidacloprid (IMI), sulfoxaflor (SULF), thiacloprid (THIAC) and thiamethoxam (THIAM) after single oral dosages at the currently ADI levels of the EFSA - Conducted for each of the seven NNIs in a single volunteer (male, Caucasian, 50 years, 83 kg body weight, non-smoker) - Doses were applied orally - Post-dose urine samples were collected consecutively and completely up to 48 h - Parent compound and two putative metabolites, N-desmethyl-ACE (DME-ACE) and 6-CNA-gly, were detected - Unchanged ACE was detected up to 24 h post-dose in urine - DME-ACE appeared in the first urine sample after dosing (3.9 h) and remained at more or less constant levels up to 48 h post-dose - Observed excretion kinetics of ACE and DME-ACE suggest that ACE is rapidly converted into DME-ACE before excretion and proof a high capacity of oxidative N-demethylation in humans - Putative 6-CNA-gly (tR = 6.2 min) could be detected in both ionization modes and a high isotopic 35Cl/37Cl correlation could be found but could not be confirmed through dd-MS - Results suggest that ACE is almost exclusively metabolized to DME-ACE in humans - Results are in line with (Harada et al., 2016)
(Wrobel et al., 2023)		- Quantitative investigation of the human metabolism and urinary excretion kinetics of seven globally important NNIs, i.e., acetamiprid (ACE), clothianidin (CLO), flupyradifurone (FLUP), imidacloprid (IMI), sulfoxaflor (SULF), thiacloprid (THIAC), and thiamethoxam (THIAM) - Urine sample from (Wrobel et al., 2022) were used, two male volunteers (37 years, 98 kg; 48 years, 96 kg) were additionally administered individual single oral doses of selected NNIs (ACE, CLO, FLUP, and IMI) - For ACE and DME-ACE the Cmax was 20 ± 7 and 520 ± 232 µg/g creatinine, respectively, two non-specific metabolites (6-CNA and 6-CNA-gly) had Cmax values of 1.3 ± 1.1 and 142 ± 97 µg/g, respectively - Tmax for ACE and DME-ACE was 4.3 ± 4.0 and 8.1 ± 4.0 h, respectively, values for t1/2 were 2.5 ± 1.2 and 19.1 ± 0.3 h, respectively - For ACE, a Fue of 0.48 ± 0.24 % was calculated after 24 h and 0.48 ± 0.25 % after 48 h - For DME-ACE Fue were 33 ± 6% and 54 ± 6% after 24 and 48 h, respectively - Based on the Fue for DME-ACE a median daily intake of 0.03 µg/kg bw/d was calculated for ACE

Reference	Cited in	Findings
(Wrobel et al., 2024)		- Case study on male volunteers after applying an ACE-containing spray agent (5 g/L; 0.5wt.–%) on ornamental plants without dermal protection, (final concentration of the active ingredient: approximately 50 mg) according to the manufacturer's instructions - Urine samples were collected directly before the application of acetamiprid (*t*0) and consecutively and completely during the following 48h - ACE itself was not quantifiable above the LOQ in any of the samples, its metabolite DME-ACE was already quantifiable in the *t*0 sample and continuously until 44 h after spraying - In the last two samples, DME-ACE was below the LOQ - The maximum measured concentration (Cmax) was 0.32 µg/g creatinine
(F. Li et al., 2022)		- To understand the reliability of a single measurement of urinary neonicotinoid biomarkers in representing a true longer-term average exposure, the temporal variability of 14 neonicotinoids and/or their metabolites over one year in 114 Chinese young adults were evaluated - Among others, DME-ACE required a minimum of 2–4 spot urine samples over one year to obtain a reliable exposure evaluation. - The authors recommended at least 2–4 spot urine samples to be used to assess 1-year neonicotinoid exposure and to consider seasonal variations when scheduling urine sample collection - 757 samples were available, ACE was detectable in 18% of them, its metabolite in 100%, with adjusted mean values of 0.11 and 3.53 µg/g creatinine
(Chen et al., 2020a)		- Nationwide survey from 23 Chinese provinces with 97 pooled breast mild samples from 3570 lactating women from 2017-2019 - DME-ACE was the most predominant compound, accounting for 61.2% of the total amount of neonicotinoids, followed by imidacloprid with only 15.6% - Concentration of acetamiprid and its metabolite in breast mild was positively correlated with corresponding dietary exposure - The detection frequency in the 97 pooled samples was 71.1% for ACE and 100% for ACE-DE with average concentrations of 19.1 (<LOD-230) and 161 (8.3-1479) ng/L, respectively
(Chen et al., 2020b)		- Dietary exposure to neonicotinoids of the Chinese adult population on the basis of 528 (240 and 288 from the 5th (2009–2012) and 6th (2015–2018) Chinese total diet studies) composite dietary samples from 24 Chinese provinces - Of the 240 samples, 52.9% were contaminated with ACE, 31.3% with ACE being detected in all 12 food categories with mean concentrations ranging from 0.011 to 26.618 µg/kg, and ACE-DE detected in 11 categories with mean concentrations ranging from 0.007 to 2.341 µg/kg - Of the 288 samples 56.9 % contaminated with ACE and 44.8% with ACE-DE - In this study, IMI and ACE were the frequent detected substances
(Chen et al., 2021)		- 120 human serum samples were collected in Wuxi city, Eastern China - Acetamiprid was one of four neonicotinoids with a high detection frequency of 12.5% and a concentration range from <LOD-194 pg/mL - No metabolites were measured, only the parent compounds
(Laubscher et al., 2022)		- Cerebro-spinal fluid (CSF), plasma and urine of 14 children from 3-18 years was analysed for different neonicotinoids from August to December 2020

Reference	Cited in	Findings
		- All CSF was positive for at least one compound - 13 (93%) CSF and urine samples and 12 plasma samples were contaminated with ACE-DE above the LOQ with a median CSF concentration of 0.0123 ng/mL (0.0024-0.1068 ng/mL) - A statistically significant linear relationship was found between plasma/urine and CSF N-desmethyl-acetamiprid concentrations
(Li et al., 2020a)		- 25 patients with osteoarthritis from Saudi Arabia were examined for serum concentrations of pesticides - ACE was found in 8% of the participants with a mean concentration of 0.004 ng/mL, ACE-DE was found in 84% of the participants with mean concentrations of 0.10 ng/mL

Reference	Cited in	Findings
(Li et al., 2020b)		- Variability of neonicotinoid concentrations in single-spot and first-morning urine in 19 individuals from the US over 44 consecutive days - For ACE, the concentrations were below LOQ - ACE-DE had median concentrations of 0.272 ng/mL in single-spot and first-morning urine
(Nimako et al., 2021)		- A total of 75 human urine samples from healthy non-farming volunteers (19 males, 56 females) from Ghana aged 18-80 were examined for several neonicotinoids - ACE-DE was the mostly detected compound in 94.7% of the samples with a median concentration of 0.41 µg/L and a maximum concentration of 8.79 µg/L - ACE was detected in 2.7% of the sample with a median <LOQ and a maximum concentration of 0.08 µg/L - Estimated daily intakes of acetamiprid were 0.47 µg/kg bw/d for females and 0.91 µg/kg bw/d for males
(I. B. Ottenbros et al., 2023)		- First morning void urine samples were analysed from 105 (29-56 years, mean: 42 years, 73 females) and 295 (20-39 years, mean: 31 years, 136 females) Dutch and Swiss adults, respectively - Farmers and other employees in the agricultural sector were not included and only winter samples were used to better assess the baseline exposure - Of all pesticides, acetamiprid was the one with the highest detection rate in both groups - most frequently co-occurring pesticide biomarker patterns included different combinations of acetamiprid-flonicamid-chlorpropham
(I. Ottenbros et al., 2023)		- Assessment of exposure to pesticide mixtures in five European countries Latvia, Hungary, Czech Republic, Spain, Netherlands - 2088 urine samples from 1050 Participants (525 parent-child pairs) - Detection frequency of ACE-DE was 99% in the samples in Spain, 33% in Latvia, 94% in Hungary, 98% in Czech Republic and 93% in Netherlands which were by far the highest detection rates of all pesticides measured
(Shalaby, S.E.B. and Abdou, G.E., 2022)		- Blood samples of 150 healthy male agricultural workers from Egypt from January to June 2017 being exposed to different classes of pesticides for at least 5 to 15 years - Detection frequency of acetamiprid was 11.7% in the subjects - Worker's age did not affect the accumulation of pesticide residues in their bodies - Apparently only the parent compound was measured but not its metabolite
(Song et al., 2020)		- 275 urine samples collected from residents aged 1-91 years in 10 cities in China - DME-ACE was detected in 89% of the samples with a median urinary concentration of 0.45 ng/mL - The ratio of m-NEO to its corresponding parent NEO (m-NEO/NEO) was 37.7, indicating that the urinary m-NEO level was significantly higher than that of the corresponding parent compound
(Suwannarin et al., 2020)		- Urinary concentrations of 100 reproductive-age farmworkers from two intensive farming areas in Chiang Mai Province, Thailand, including 50 each - NEO exposure was determined by measuring the urinary concentrations of NEO compounds and their metabolites (NEO/m)

Reference	Cited in	Findings
		- The metabolite had a detection frequency of 99% with a geometric mean concentration of 7.3 µg/g creatinine - However, the max concentration measured was below the geometric mean and below the confidence interval, so the values are not trustworthy - Urinary concentrations of N-DME-ACE were significantly lower in men than in women
(Suwannarin et al., 2024)		- Urine samples from 30 pregnant women in Japan between 12 and 22 weeks of gestation on five occasions within two weeks - Most frequently detected NEO/m was DME-ACE with 95.3 % determination rate and a maximum concentration of 37.7 ng/mL, parent compound was detected in 18.0% of the probes and a maximum concentration of 1.3 ng/mL in first morning voided urine - In spot urine samples the detection rate for acetamiprid was 15.3% and 96.0% for the metabolite with maximum concentrations of 0.8 and 31.7 ng/mL, respectively
(Taira et al., 2021)		- To elucidate the relationship between renal tubular dysfunctions and urinary neonicotinoids concentrations, spot urine samples from15 chronic kidney disease (CKD) patients, their families and neighbours were collected in Sri Lanka in 2015 - DME-ACE was detected in 92.4% of the urine samples with a mean value of 0.88 ± 1.09 ng/L, followed by dinotefuran (17.4%), thiamethoxam (17.4%), clothianidin (9.8%), thiacloprid and imidacloprid - Detection levels in the CKD patients were lower than in the non-CKD participants
(Ueyama et al., 2020)		- Urine samples from 50 3-year-old diapered children (31 males, 19 females) from Japan were collected - The detection rates of Acetamiprid and its major metabolite were 10 and 78%, respectively, the maximum concentrations were 0.7 and 14.56 µg/L - The geometric mean of the metabolite was 0.34 µg/L, for acetamiprid no gm could be calculated - The paper was rather about the development of an analytical method
(A. Wang et al., 2020)		- 408 urine samples of 129 healthy adults from Wuhan, China during autumn and Winter 2018 - Expectantly, the amounts of the metabolites were higher than that of the parent compound - Detection frequency of ACE and DME-ACE were 38.0 and 96.9%, respectively - The concentration ranges were below the detection limit until 0.93 ng/mL for ACE and until 18.3 ng/L for DME-ACE, geometric mean was 0.02 and 0.7 ng/mL, the mean values were 0.05 ± 0.13 and 1.66 ± 2.47 ng/mL, respectively - According to the authors, DME-ACE can be used as biomarker for human biomonitoring of IMI
(H. Wang et al., 2020)		- 309 school children aged 7–11 years were selected from a dynamic cohort of children established in Shanghai, East China in 2019 - Eight neonicotinoids and four metabolites were determined in first morning urine - Acetamiprid and its metabolite were detected in 1 and 62.6 % of the probes, respectively

Reference	Cited in	Findings
		- Some demographic and socioeconomic factors, physical exercise, and BMI were found to be predictors for the exposure to neonicotinoids - Tap water and fresh vegetables were found to be potential exposure sources.
(Yang and Liang, 2022)		- Relationship between NEOs metabolites and hematologic parameters using a cross-sectional study design in 1397 US adults of the National Health and Nutrition Examination Survey (NHANES) 2015–2016 - Levels of NEOs metabolites in urine and hematologic markers were measured - Detection rate of DME-ACE was 32.64% which is rather low when compared to other studies and the maximum concentration was 14.6 μg/L - DME-ACE was related to reduced white blood cells (WBC) (β = − 0.419; 95% CI: − 0.764, − 0.074) and neutrophil counts (β = − 0.349; 95% - CI: − 0.623, − 0.074) but significantly inversely associated with platelet counts
(Henglin Zhang et al., 2022)		- Evaluation of prenatal exposure to NEOs and determination of transplacental transfer rates of NEOs from the mother to foetus determined in 95 paired maternal (MS) and cord serum (CS) samples collected in southern China - All participants were healthy, without having occupational exposure to NEOs, age: 18–37 years, mean: 25.8 years, duration of pregnancy: 38–43 weeks, mean: 41.8 weeks - Detection rate of ACE and ACE-De was 59% and 85% in cord serum samples, and 67% and 71% in maternal serum samples, respectively - Mean values were 0.028 and 0.3 ng/mL in cord serum and 0.032 and 0.27 ng/mL in maternal serum, respectively - Median transplacental transfer efficiencies (TTEs) of ACE (1.44) and its metabolite (1.26) indicate indicating efficient placental transfer
(Mahai et al., 2022)		- Nine parent NNIs and ten main metabolites in 1224 urine samples donated by 408 pregnant women at three trimesters were measured - The unadjusted vs. specific gravity (SG) adjusted median concentrations and detection frequencies (DFs) of desmethyl-acetamiprid (1.01 vs. 1.08 ng/mL; DF: 99.7%), was higher than corresponding parent NNI, acetamiprid (ACE; <0.01 vs. <0.01 ng/mL; 26.4%) - A decreased trend was observed in urinary NNIs and their metabolites throughout the three trimesters, maternal age, educational level, and household income were related to the concentrations
(H. Zhang et al., 2023)		- Between January and March of 2018, urine and whole blood samples from infants, in addition to breast milk, infant formula, and tap water collected in South China (97 infants' whole blood, 103 infants' urine (first morning void), 22 breast milk, and 18 infant formula) - Detection rate of ACE was 92%, 92%, 55% and 67% in infants' urine, infants' whole blood, breast milk and dissolved infant formula, respectively with median values of 0.031, 0.1, 0.015 and 0.018, respectively - For ACE-DE the detection rate was 64%, 79%, 73% and 0% in infants urine, infants' whole blood, breast milk and dissolved infant formula, respectively with median values of 0.015, 0.041, 0.041 and<LOQ, respectively - Older infants (181–360 days) might face higher NEO and DME-ACE exposure than younger infants (0–180 days)

Reference	Cited in	Findings
		- According to the authors, blood samples contained a significantly ($p < 0.01$) higher median concentration than that of urine samples suggesting that NEOs readily partition into blood
(Q. Zhang et al., 2023a)		- 115 adult whole blood and plasma specimens from individuals living in Anji, Zhejiang, China, participated in this study from April to May 2018 for analysis of eight neonicotinamides to analyse partitioning and distribution in blood samples - Partitioning capacity of ACE among plasma and red blood cells is comparable but no information on its metabolite in the study
(Zhao et al., 2022)		- First morning urine samples of 305 elementary school children aged 8 – 11 in Shenzhen, China - Detection frequency of ACE was 95.1 % with a median concentration value of 0.01 µg/L - No metabolites were included in this study
(Thompson et al., 2023)		- Private-well tap water and human urine samples of 50 participants in a region of intense agriculture overlying vulnerable aquifers in eastern Iowa - Estimated daily intake via water of NEOs was 0.004, 0.001, 0.001, 0.001 and 0.001 ng/kg bw/d for acetamiprid in infants, toddlers, children, teenagers and adults, respectively, the maximum EDI values were 0.004, 0.001, 0.001, 0.001 and 0.001 ng/kg bw/d - In the urine samples, acetamiprid had a detection rate of 9% with a maximum concentration of 0.1 µg/g creatinine - For ACE-DE the detection frequency was 89% with a maximum concentration of 3.4 µg/g - According to the authors, the study results indicate that while the consumption of private well tap water provides a human exposure pathway, the companion urine results provide evidence that diet and/or other exposure pathways (e.g., occupational, house dust) may contribute to exposure more than water contamination but further biomonitoring research would be needed

Table IX.74: In vitro studies with human cell lines

Reference	Cited in	Findings
(Cheng et al., 2020)	NTP 2020	- Combined toxicity of imidacloprid (IM), acetamiprid (ACE) and thiamethoxam (TH) using human neuroblastoma cell line (SK-N-SH) and lepidopteran cell line (Sf-9) - Binary and ternary mixtures could enhance the inhibition of growth of both SK-N-SH and Sf-9 cells at low concentrations with relatively higher inhibitory effects on Sf-9 cells than on SK-N-SH cells - TH had higher toxicity than IM and AC - BMDL10 values of different combinations were all lower than those for individual compounds, the complex of ACE and TH had the highest combined toxicity with lowest BMDL10 value

9.3 Animal findings

Table IX.75: Overview on toxicokinetics and metabolism in animals

Reference	Cited in	Findings
Sheets et al. 2001	(Anadón et al., 2020)	• It was found in a male mouse study that acetamiprid can readily cross the blood-brain barrier, and the concentrations were detectable in the brain at 60–70% of the serum within 15min after a single intraperitoneal injection of 10mg/kg bw • The chemical analysis was also performed in the liver and plasma at 15–240min and 0–24 h in urine. The highest concentrations of the parent compounds were observed in the brain (11–16µg/g for nitenpyram and thiacloprid, 6µg/g for imidacloprid, and 3µg/g for acetamiprid). Significant concentrations of acetamiprid achieved in the tissues were higher than the other neonicotinoid
Premkumar, Guo and Vegurlekar 1995	(FAO and WHO, 2012)	• SD rats (3/sex/group) orally exposed to 1 mg/kg bw/d [14C]acetamiprid (purity: 99.9%) for 15 days, Group 1-3 (3/sex/group) were sacrificed 1, 10 or 96 h after dosing, groups IV and V (5/sex/group) 96 h after being exposed to unlabelled acetamiprid for 14 days following a single dose of radioactively labelled acetamiprid, study performed according to GLP • Blood drawn from rats of group III 1 h post-dosing on days 1, 3, 7, 15 with average conc. in blood of 0.477-0.747 and 0.465-0.698 µg/mL in males and females, respectively, from rats of group V at 0.25, 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, 48 h with Cmax of 0.798 ± 0.111 µg/mL, *T*max of 2.80 ± 0.637 hours, absorption half-life ($t_{½}$ (ka)) of 1.35 ± 0.825 hours and AUC∞ of 8.35 ± 1.12 µg eq·h/mL in males and Cmax of 0.861 ± 0.132 µg/mL, *T*max of 2.81 ± 0.894 hours, absorption half-life ($t_{½}$ (ka)) of 1.18 ± 0.868 hours and AUC∞ of 10.3 ± 2.90 µg eq·h/mL in females • Elimination half-lives ($t_{½}$ (k)) for the male and female rats were 4.42 ± 1.10 hours and 5.56 ± 1.93 hours, respectively • Pharmacokinetic parameters for both sexes did not differ considerably, Tmax values indicate rapid rate of absorption with a maximum blood concentration within 2-3 hours • Acetamiprid was rapidly absorbed (>90% within 1h) from the GI tract with most of it (53–65%) was excreted in the urine, excretion in urine and cage rinse combined amounted to 61-73% • No difference in elimination of test substance between chronic administration for 14 days followed by a single administration of radioactively labelled acetamiprid on day 15 (group IV) and chronic administration of radioactively labelled acetamiprid for 15 days (groups I, II and III) • Amount of administered radioactivity eliminated in faeces was lower for females (22–29%) than for males (30–35%) • Whole blood, liver, kidney, lung, pancreas, spleen, heart, brain, testes (male), ovary (female), skeletal muscles, inguinal fat (white), skin with hair, thyroid, bone, adrenal glands, gastrointestinal tract with contents, cage rinses and residual carcasses were collected from each rat of groups I, II, III and IV • Radioactivity, after administration of the last chronic dose, was detected at the earliest sampling point (1 hour) in all the tissues collected from each rat being highest at 1 hour post-dosing in most tissues and declining rapidly thereafter (groups II and III)

Reference	Cited in	Findings
		• Residue levels seen in tissues collected 10 hours post-dosing (group II) substantially lower than in tissues collected 1 hour post-dosing • Elimination half-life (t½(k)) for both sexes indicated rapid elimination • Residue levels seen in tissues collected 96 hours post-dosing (group III) very low compared with the tissues collected 1 and 10 hours post-dosing • Elimination half-life (t½(k)) for both sexes was between 4 and 6 hours postdosing, indicating that the rate of elimination was rapid and that retention of residue in tissues after chronic administration was minimal • Highest radioactivity levels observed in the Gi tract, liver and kidney in both sexes at all sacrifice times, lowest concentration in bone and white fat • Residue levels higher in all tissues of rats chronically treated with [14C]acetamiprid for 15 days (group III) compared with the rats in group IV, with a single final dose of [14C]-acetamiprid following 14 days of non-labelled acetamiprid doses. • Residue levels observed in the tissues of rats sacrificed 96 hours after the last dose were very low (0.01–0.1 ppm) as most of the administered dose (> 90%) was eliminated through the urine and faeces • Total administered radioactivity recovered in groups I - IV in the range of 91.7–106%, whereas recovery in group V (the pharmacokinetics group) was 71.7% and 85.6% in males and females, respectively
Tanoue and Mori 1997a	(FAO and WHO, 2012)	• GLP compliant study to investigate absorption, distribution, metabolism and excretion of acetamiprid in rats • [Pyridine-2,6-14C]acetamiprid administered i.v. or orally to rats (5/sex/dose) in groups A, B and CN-B at 1.0 mg/kg bw in group D at 50 mg/kg bw • Group A for determination of absorption rate, Groups B, D and CN-B for blood levels, tissue distribution, metabolite analysis and excretion rate • Absorption rate in groups B and D was rapid: Tmax was 0.5-2 h at 0.91 mg/kg bw for males and 1.01 mg/kg bw for females (group B) and 3-7 h after 40.6 mg/kg bw for males and 31.46 mg/kg bw for females (group D) • Absorption rate after oral administration was >95% (days 0-1: 96.7%/96.3% for m/f, days 0-4: 99.4%/99.5% for m/f) when comparing urinary excretion rate of oral to urinary excretion rate of i.v. exposure • After Cmax in blood, levels decreased linearly and rapidly with half-lives of radioactivity being 5.84–7.11 h for group B and 8.07–15.03 h for group D, absorption in group CN-B was rapid, with Cmax in the blood of 0.97 mg/kg for both sexes and tmax 1–2 hours, then decreasing linearly and rapidly, with t1/2 of the radioactivity of 5.90–11.29 hours • In group A, excretion rates 1 day after dosing in the urine were 76.00–78.92% and in faeces 11.44–11.90%, in group B 73.16–76.28% and 9.91–11.10%, in group CN-B were 75.15–79.24% and 4.12–4.56% in urine and faeces, respectively, In group D, 72.84% and 56.39%, in urine and 6.13% and 10.20% in faeces of males and females, respectively • Total excretion rates in all groups >90% with 97.78%/97.26%, 93.13%/93.64%, 100.13%/91.74%, 96.55%/94.38% for groups A, B, D, CN-B for males and females, respectively • Tissue concentrations in groups B and D were investigated 1, 5, 10 and 96 hours and 5, 14, 24 and 96 hours after administration: shortly after dosing radioactivity was widely distributed, concentrations in bone and fat clearly

Reference	Cited in	Findings
		low compared with blood, adrenal (group B: 1.344–2.409 mg/kg at 1 hour; group D: 51.88–62.87 mg/kg at 5 hours), thyroid (group B: 1.345–1.493 mg/kg at 1 hour; group D: 64.72–68.13 mg/kg at 5 hours), liver (group B: 1.651–1.711 mg/kg at 1 hour; group D: 61.34–62.02 mg/kg at 5 hours) and kidney (group B: 1.458–1.777 mg/kg at 1 hour; group D: 52.55–55.66 mg/kg at 5 hours) had higher concentrations than the blood (group B: 0.771–0.803 mg/kg at 1 hour; group D: 31.46–34.77 mg/kg at 5 hours) • Blood concentrations in groups B and D were 0.001 mg/kg and 0.07 mg/kg, respectively, after 96 hours • Half-lives of the radioactivity in the tissues ranged from 2.9 to 7.9 hours (group B) and from 6.0 to 8.5 hours (group D) with no tissue accumulating the substance • In summary, acetamiprid orally dosed in rats was rapidly absorbed and widely distributed into the tissues via blood with the majority of the radioactivity being excreted in the urine and in the faeces. Disappearing rapidly from the body with no tissues that are presumed to accumulate the compound and no differences in the sexes
Premkumar and Guo 1995	(FAO and WHO, 2012)	• GLP compliant biliary excretion study in 4 male and 4 female bile duct-cannulated SD rats after single dosing of [14C]acetamiprid (purity: 97.1%) via gavage at 1.02 and 1.07 mg/kg bw for males and females, respectively • Steady increase in [14C]acetamiprid residue level in bile from 3 to 12 hours post-dosing with tmax after 12 h in males and females • Average recovery of the administered dose in bile over a 48-hour period was 19.9% ± 1.47% in males and 18.6% ± 0.62% in females accounting for less than 20% of total dose administered • Absorption of the test substance and extent of first-pass metabolism not significantly different between both sexes • Average recovery of the administered dose in faeces over a 48-hour period was 6.72% ± 3.36% in males and 5.84% ± 0.86% in females • Average recovery of the administered dose in urine over a 48-hour period was 24.3% ± 5.22% in males and 36.9% ± 3.80% in females • Sum of urine plus cage rinses were 60.2% ± 5.20% and 64.4% ± 2.86% for males and females, respectively, accounting for the major residues • Average recovery of the administered dose in liver at 48 hours post-dosing was 0.22% ± 0.13% in males and 0.18% ± 0.18% in females • Average recovery of the administered dose in Gi tract at 48 hours post-dosing was 0.46% ± 0.34% in males and 0.33% ± 0.23% in females • Total recoveries of the administered dose in the three male rats were 93.2%, 92.8% and 89.6%, respectively, in the three female rats 94.9%, 93.5% and 91.2%, respectively
Cheng 1997	(FAO and WHO, 2012)	• Extent of absorption of acetamiprid was studied following application of 70% wettable powder containing [14C]acetamiprid (purity 97.5%) to the skin of male Crl: CD(SD)BR rats investigated in a GLP compliant study with dose levels • Preliminary phase with 2 groups of 4 rats/group conducted to evaluate and establish test material application and skin washing techniques with dermally dosing at two levels of 0.0128 mg/animal (1.01 µg/cm^2) and 1.26 mg/animal (101 µg/cm^2)

Reference	Cited in	Findings
		• Definitive phase with 3 groups of 24 rats/group were dermally dosed with [14C]-acetamiprid at three dose levels of 0.0136 mg/animal (1.09 µg/cm^2), 0.119 mg/animal (9.53 µg/cm^2) and 1.13 mg/animal (90.2 µg/cm^2) • Four rats per time point from each dose group were sacrificed at 0.5, 1, 2, 4, 10 and 24 hours • Mean total recovery of radioactivity ranged from 96.6% to 102%, with most of the radioactivity (63.9–87.5%) in the skin wash (radioactivity in the skin at the application site accounted for 10.2–32.2% of the applied radioactivity) • Radioactivity in blood, excreta and carcasses accounted for less than 6.50% of the applied radioactivity • Amounts of dermal absorption increased with increasing exposure time; highest absorption at 24 hours post-dosing, accounting for 4.27% (0.581 µg), 6.34% (7.54 µg) and 2.82% (31.9 µg) for the 1.09, 9.53 and 90.2 µg/cm^2 dose groups, respectively • Highest concentration of radioactivity in blood was 0.001 ppm at 1.09 µg/cm^2 24 hours post-dosing, 0.019 ppm and 0.010 ppm at 9.53 µg/cm^2 10 and 24 hours post-dosing, respectively, and 0.041 ppm for the 90.2 µg/cm^2 dose group at 24 hours post-dosing
Premkumar, Guo and Vegurlekar 1995	(FAO and WHO, 2012)	• Group IV animals of the Premkumar, Guo and Vegurlekar (1995) study used for quantitative and qualitative GLP compliant analysis of metabolites • Major radioactive compounds in the excreta of rats were acetamiprid itself (males: 5.21%; females: 7.41%), demethylated compound IM-2-1 (males: 15.48%; females: 20.39%), nicotinic acid derivative IC-O (males: 11.12%; females: 8.01%) and IC-O glycine conjugate IC-O-Gly (males: 10.10%; females: 10.32%) • In addition, MeS-IC-O, IM-1-4, IM-2-4, IM-O, IM-1-3 and IM-2-3 were detected, accounting for less than 2% of the dose • Several unknown compounds in urine, with maximum abundance of an unknown compound being 1.0% • Major metabolic routes of acetamiprid in rats are production of IM-2-1 by *N*-demethylation, the production of IC-O by detachment of the cyanoacetamide side-chain from IM-2-1, and the production of IS-1-1 and IS-2-1 by detachment of the cyanoacetamide sidechain from acetamide and IM-2-1, respectively
Tanoue and Mori 1997b	(FAO and WHO, 2012)	• Radioactive compounds from the study of Tanoue and Mori (1997a) in the excreta of rats were identified and quantified • Major compounds identified were acetamiprid itself (males: 6.10%; females: 5.63%), demethylated compound IM-2-1 (males: 19.51%; females: 19.00%) and nicotinic acid derivative IC-O (males: 28.19%; females: 25.52%) in group B; acetamiprid (males: 7.75%; females: 7.34%), IM-2-1 (males: 24.48%; females: 21.37%) and IC-O (males: 27.11%; females: 27.63%) in group D; and acetamiprid (males: 4.16%; females: 6.12%), IM-2-l (males: 13.39%; females: 18.98%) and IC-O (males: 28.13%; females: 24.73%) in group A; acetamiprid (males: 3.98%; females: 4.51%), IM-2-1 (males: 16.95%; females: 16.56%), IS-1-1 (males: 13.15%; females: 16.45%) and IS-2-1 (males: 35.61%; females: 30.23%) in group CN-B • IC-O-Gly, MeS-IC-O, IM-1-4, IM-2-4, IM-O, IM-1-3 and IM-2-3 detected in groups A, B, D at less than 4% of the dose

Reference	Cited in	Findings
		• Main metabolic pathways of acetamiprid in rats were the transformation to IM-2-1 by demethylation and further to IC-O after cleaving IS-1-1 and IS-2-1 from acetamiprid and IM-2-1, respectively
Saito 2003	(FAO and WHO, 2012)	• GLP compliant study to investigate whether metabolite IM-1-5 was found in excreta after male rats (3/group) were gavaged with a single dose of 1 or 51 mg/kg bw • Excretion was rapid with most of the radioactivity (85.7% for LD within 24 hours and 90.4% for HD within 48 hours) was eliminated mostly in the urine • Amount of IM-1-5 accounted for 4.5% and 0.4% of the initially administered radioactivity in LD and HD urine, respectively • No IM-1-5 detected in faeces • Major metabolite in excreta was IC-O (35.9% and 33.6% at LD and HD, respectively, IM-2-1 (18.5% and 9.3% at LD and HD, respectively and Acetamiprid (5.2% and 4.5% at LD and HD)
(Khidkhan et al., 2021)		• Study focused on the differences in metabolites of neonicotinoids (imidacloprid, clothianidin and acetamiprid) and CYP activities among cats, dogs, humans, and rats (in vitro). • CYP activities in metabolism of acetamiprid to DME-ACE and N-acetyl-acetamiprid were determined to be significantly higher in humans compared to other species.
(Kolanczyk et al., 2020)		• Study to identify and compare neonicotinoid metabolic pathways of in vitro microsomal systems in the rainbow trout and rat • Kinetics for the microsomal demethylation of acetamiprid to DME-ACE were determined in the rat (Km = 70.9 µM; Vmax = 10 pmoles/min/mg)
(Shinya et al., 2022)		• In vitro assessment of the ability of raccoons to metabolize NNIs using hepatic microsomes • Acetamiprid concentrations 10, 25, 50, 100, 200, and 400 µM • In vitro acetamiprid metabolism assay confirmed the formation of dm-acetamiprid in both raccoons (1.1+-0.6) and rats (4.3+-1.3) (Michaelis–Menten kinetics parameters)
(Shinya et al., 2023)		• The cytochrome P450 (CYP)-dependent metabolism affected by acetamiprid • Blood concentration of ACP decreased more quickly in musk shrews than in mice • Dm-ACP, N-ace-ACP, and N-ac-dm-ACP were detected among the metabolites • N-ace-ACP and N-ace-dm-ACP were detected exclusively in mice and at very low concentration levels (Cmax = 7947 ±251 ng/mL [dm-ACP], 2.44 ± 0.38 ng/mL [dc-ACP], 6.34 ± 1.53 ng/mL [N-ace-ACP], and 10.92 ± 1.47 ng/mL [N-ace-dm-ACP]). • Musk shrews showed significantly higher maximum concentrations of ACP (Cmax = 11,940 ± 793 ng/mL) than mice (Cmax = 7947 ± 251 ng/mL) (p = 0.012). • A significantly lower half-life was observed in musk shrews (T1/2 = 93 ± 9 min) than in mice (T1/2 = 153 ± 12 min) (p = 0.007). In dm-ACP, musk shrews showed significantly higher AUC than mice (musk shrew: 5452 ± 748 µg·h·mL^{-1}, mice: 4427 ± 671 µg·h·mL^{-1}) (p = 0.024).

Reference	Cited in	Findings
		• A high blood concentration of dm-ACP indicated that this is the main metabolite produced by ACP both in musk shrews and mice. Michaelis–Menten kinetic parameters

Acetamiprid is classified as toxic if swallowed. It has a low dermal and inhalation toxicity.

Table IX.76: Overview acute toxicity studies

Reference	Cited in	Route of Exposure	Species	Results (LD50/LC50)
Sheets et al. 2016	(Zuščíková et al., 2023)	Oral	Rat	182 mg/kg bw
Gasmi et al. 2017, Kong et al. 2017, Shamsi et al. 2021	(Zuščíková et al., 2023)	Oral	Rat	200-220 mg/kg bw
Hataba et al. 2014, Shahin et al. 2018	(Zuščíková et al., 2023)	Oral	Rat	1000 mg/kg bw (drinking water)
EC 2018	(Anadón et al., 2020)	Oral	Rat	195 mg/kg
RAR 2015 Fujii (2002a)	(FAO and WHO, 2012; RAC Opinion, 2020)	Oral	Rat (Crj:CD(SD), IGS, SPF)	Males: 195 mg/kg bw, Females: 140-200 mg/kg bw
RAR 2015 Takaori (1997b)	(FAO and WHO, 2012; RAC Opinion, 2020)	Oral	Rat (Crj:CD(SD), SPF)	Males: 417 mg/kg bw Females: 314 mg/kg bw
RAR 2015 Mochizuki and Kanaguchi 1992	(FAO and WHO, 2012; RAC Opinion, 2020)	Oral	Rat (Crj:CD(SD), SPF)	Males: 217 mg/kg bw, Females: 146 mg/kg bw
Mochizuki and Goto 1992	(FAO and WHO, 2012)	Oral	Mouse (Crj:ICR,SPF)	Males: 198 mg/kg bw, Females: 184 mg/kg bw
Redgrave 1994	(FAO and WHO, 2012)	Oral	Birds (hybrid brown laying strain Gallus gallus domesticus)	129 mg/kg bw
Mochizuki & Fujii (1998)	(FAO and WHO, 2012)	Dermal	Rat (Crj:CD(SD), SPF)	Males and females: > 2000 mg/kg bw
Takaori (1997a)	(FAO and WHO, 2012)	Dermal	Rat (Crj:CD(SD), SPF)	Males and females: > 2000 mg/kg bw
EC 2018	(Anadón et al., 2020)	Dermal	Rat	> 2000 mg/kg bw
Saika (1994)	(FAO and WHO, 2012)	Inhalation	Rat (Crj:CD(SD))	Males and Females: >0.3 mg/L, MMAD: 5 µm

Reference	Cited in	Route of Exposure	Species	Results (LD50/LC50)
Jackson (1997)	(FAO and WHO, 2012)	Inhalation	Rat (SD)	Males and Females: >1.15 mg/L, MMAD: 8 µm
EC 2018	(Anadón et al., 2020)	Inhalation	Rat	> 1.15 mg/L
Goto 1997	(FAO and WHO, 2012)	Oral	Rat (Crj:CD(SD)IGS)	Metabolite IM-1-5: males: 119 mg/kg bw, females 104 mg/kg bw
Fujii 2002b	(FAO and WHO, 2012)	Oral	Rat (Crj:CD(SD)IGS)	Metabolite IM-1-5: males 141 mg/kg bw, females: 132 mg/kg bw
Mochizuki and Goto 1994a	(FAO and WHO, 2012)	Oral	Rat (Crj:CD(SD))	Metabolite IM-1-2: >5000 mg/kg bw
Kanaguchi 1999a	(FAO and WHO, 2012)	Oral	Rat (Crj:CD(SD)IGS)	Metabolite IB-1-1: >2000 mg/kg bw
(Sokolyuk et al., 2020)		Oral	Mice (not specified)	Mospilan RP: active substance 131.25 mg/kg bw, drug: 656.25 mg/kg bw

Table IX.77: Overview of neurotoxicity studies

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Hughes 1997a	(FAO and WHO, 2012)	Crl:CD(SD)BR rats (3/sex/dose) Dose range-finding study for assessment of behavioural changes	10, 50, 100	Single exposure (purity 99.9%), examination 14 days after dosing		Clinical signs of dilated pupil (females only) and tremors and initial impairment in bw gain, body tremors, chewing (females only), hunched posture and clear reduction in body temperature at HD Body tremors and tail tremors (females only) at MD Time for peak effect was between 5 and 6 hours post-dosing
Hughes 1997b	(FAO and WHO, 2012)	Crl:CD(SD)BR rats (10/sex/dose) (GLP)	0, 10, 30, 100	Gavage, single exposure (purity 99.9%), examination 6h, 7d, 14 d after dosing	NOAEL: 10	Sign. increased tremor, hunched posture, unsteadiness and coldness to touch at HD Food consumption and bw gain in HD males significantly decreased No remarkable differences in functional observation Chewing movements of the mouth observed in one HD male one LD female, five HD females (stat. significant) Dilated pupils in 3 HD males, 6 HD females Increased frequency of urination in MD and HD males (significant in HD) Increased incidence of walking on toes in HD males (sign.) and all females 2 HD females unable to lift their front feet Incidence of defecation was statistically significantly increased in HD males, not attributed to treatment Sign. increased forelimb grip strength in HD males, slightly increased in females Lower splay values in HD males and females after 5 hours Coulbourne activity sign decreased in MD and HD males and HD females after six h (basically no activity at HD)

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						No microscopic findings that were considered to be treatment related Main effect of treatment appears to be cns with observation of tremors and effects on autonomic nervous system by increased urination, increased pupil dilation, increased muscle tone, no apparent effects on sensory systems, no evidence of neuropathology
Hughes 1997c	(FAO and WHO, 2012)	Crl:CD BR rats (10/sex/dose) (GLP)	0, 100, 200, 800, 1600 ppm (7.4, 14.8, 59.7, 118 mg/kg bw/d for males and 8.5, 16.3, 67.6, 134 mg/kg bw/d for females	Dietary exposure (purity 99.9%), 13 weeks	NOAEL: 200 ppm (14.8 and 16.3 mg/kg bw/d for males and females, respectively)	Functional observational battery prior to treatment and after 4, 8 and 13 weeks of treatment No increased mortality Quantitative assessment of locomotor activity unaffected No differences in brain parameters No findings in neuropathology Decreased bw and food consumption at 800 ant 1600 ppm No behavioural changes being indicative of neurotoxicity
Nemec 2004	(FAO and WHO, 2012)	Crl:CD(SD)IGS BR rats (12/dose) (GLP)	0, 5, 10, 20, 40	Gavage (purity 100%) GD6 to lactation day 10	NOAEL for maternal tox: 10 NOAEL for development: 20 NOAEL for neurotox: 40	No increased mortality General physical conditions unaffected Duration of gestation unaffected Mean bw gain and food consumption decreased from 20 mg/kg bw/d Mean numbers of pups born, mean live litter size, percentage of males per litter at birth and postnatal survival were unaffected HD mean pup weights were reduced during PND 4, 7 and 10 General physical condition of F1 pups during postnatal period unaffected

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						Necropsy findings of pups found dead or at scheduled euthanasia on PND21 were normal No observations indicative of neurotoxicity in either the maternal animals or the F1 offspring
Nemec 2003	(FAO and WHO, 2012)	Crl:CD(SD) IGS BR rats (25/dose) (GLP)	0, 2.5, 10, 45	Gavage (purity >99%) GD 6 to lactation day 21	NOAEL: 2.5 for maternal tox, developmental tox and developmental neurotox	One HD female died on GD23 due to dystocia No clinical sings of toxicity Mean bw loss, decreased bw and food consumption in HD, but bw gain increased on lactation days 1-4 No findings at necropsy of F0 females Mean numbers of implantation sites similar to ctrl Mean live litter size 7.3% lower than ctrl in HD and decreased postnatal survival on PND0-1 Total litter loss in three HD females General physical condition of F1 animals normal Offspring bw gains sign. Decreased on PND1 in HD males and females Mean numbers of days to acquisition of balanopreputial separation and vaginal patency not affected For auditory startle response, the maximum response amplitudes (*V*max) and average response amplitudes (*V*avg) were reduced in HD and MD males Locomotor activity, learning and memory in all offspring were unaffected F1 pups necropsy not affected No microscopic findings in the central and peripheral nervous system tissues examined from the selected PND 11 and PND 72 pups Mean absolute and relative brain weights, brain lengths, brain widths and F1 brain morphometry at PND 11 and PND 72 not affected

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Redgrave 1994	(FAO and WHO, 2012)	Hybrid brown laying strain Gallus gallus domesticus birds (12/group) (GLP)	0, 129 (LD50 value)	Oral (not specified, purity >99%) single exposure, observation for 14 days	NOAEL for neurotoxicity: 129	No clinical signs of delayed neurotoxicity when assessed in terms of ataxia No histological evidence of acute delayed neurotoxicity No other treatment-related changes were detected No significant reductions in brain acetylcholinesterase levels or neuropathy target esterase levels in the brain and spinal cord
EFSA 2013, EC 2018	(Anadón et al., 2020)	Rats	10, 30 and 100	Oral, single exposure, acetamiprid	10	Decreased locomotor activity observed Tremors and chewing movements were observed among males and females treated at 100mg/kg bw. At highest dose, males were difficult to handle, pupils were dilated in both sexes, animals felt cold to touch, and males showed increased urination. Gait and posture were also affected the day after exposure. At 30mg/kg bw, there were a few animals observed with tremors and increased urination among males
Gasmi et al. 2016, 2017	(Phogat et al., 2022)	Male Wistar Rats	3.14	Oral, 90 days, acetamiprid		Reduced GSH content and increased MDA levels Decrease of GPx and CAT activities while GST and SOD activities were increased Altered respiratory potential and membranous permeability in brain mitochondria
Khovarnagh and Seyedalipour 2021	(Phogat et al., 2022)	Male Wistar Rats (6/group)	5	Intraperitoneal, 7 days, acetamiprid		Study was conducted to investigate the protective effects of N-Acetyl-L-cysteine (NAC) and S methyl- L-cysteine (SMC) against hepatic oxidative stress and brain damage induced by acetamiprid in rats Promoted LPO (lipid peroxidation), CAT (catalase), and GST (glutathione-S-transferase) activities Induces liver injuries including infiltration of inflammatory cells, congestion and altered histo-architecture

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						Diminished TAC (total antioxidant capacity) and GSH levels in brain Gliosis, necrosis and hyperaemia in brain tissue architecture
Gasmi, 2020		Male Wistar rats (8/group)	0, 3.14	Oral, 90 days	LOAEL: 3.14	Clear perturbation of neurotransmitter levels (adrenaline, serotonin, dopamine, glutamate and AChE) either regionally or totally
Hirai et al., 2022		Male C57BL/6 J mice (10)	20	Oral, once, acetamiprid		Ca2+ imaging using a two-photon microscope to detect the abnormal activity of neuronal circuits in the brain after neonicotinoid application Increased anxiety-like behaviour associated with altered activities of the neuronal population in the somatosensory cortex Detection of acetamiprid metabolites in the brain 1 hour after chemical administration
Shamsi et al., 2021		Male Wistar rats	10, 20, 40 (10/group)	Gavage, 28 days, acetamiprid (purity 99%)		Study focused on effects of acetamiprid exposure on spatial memory (Morris water maze, "MWM") and possible vulnerability of hippocampal glutamatergic system Acetamiprid in the serum and hippocampus was measured. In addition, glutamate level and changes in the expression of NR1, NR2, and NR2B genes were measured in the hippocampus; also, the hippocampus tissue was histologically evaluated. Body weight changes were seen only in high dose group - significantly decreased on the 3rd and 4th weeks of treatment in comparison with the control group Dose-dependently increased level of acetamiprid in both the serum and hippocampus tissue after 28 days of treatment. MWM results: significant treatment and day factor effect on escape latency and travelled distance

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						parameters at 20 and 40 mg/kg doses on the first and the second days of training; low dose group did not affect learning parameters during training days. No significant difference in swimming speed parameter between control and all dose groups. Probe test – animals in all dose groups spent less time in target quadrants compared to the control group. A notable difference between the time spent in target quadrant and opposite quadrant of the control group, but no significant difference was observed in all dose groups Changes of subunit gene expression (NR1, NR2A, NR2B) in dose groups relative to the control group. Although the expression of NMDA receptor subunits relative to control group shows no significant change in low dose group, but a remarkable gene expression decrease was observed in medium dose group. A significant reduction in the hippocampus glutamate level observed in all dose groups compared to control group. In high dose group were observed morphological changes in neurons which led to a neuronal shrinkage and pyknotic nuclei, along with an increase in glial cell numbers, where dentate gyrus area of hippocampus is located. Rats' hippocampal tissue shows slight morphological change in medium dose group, but normal structure was observed in low dose group.
Von Hellfeld, 2022		Adult "Westaquarium" strain zebrafish	6.25, 12.5, 25, 100 µM (FET test) 2.50-100 µM (Coiling assay,	FET test (2-120, hours post fertilization, "hpf") Coiling assay (21-47 hpf) Swimming assay (83 – 120 hpf)		Study investigating effects of acetamiprid (and other neonicotinoid) on embryonic development and behaviour of zebrafish (Danio rerio) embryo. FET test - no severe compound-induced sublethal effects were seen at < 100 µM

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
			Swimming assay) (10 eggs per treatment group)			Coiling assay - exposure to ≥ 50 µM of acetamiprid induced significant alterations in both mean burst duration and burst count per minute (hyperactivity). induced a concentration-dependent decrease in early "number of movements/min," as well as slightly delayed development, with a complete lack of response occurring after treatment with 4.4 mM. Swimming assay - 100 µM of acetamiprid induced alterations in the frequency and extent of movements. Led to reduced swimming distance and frequency in hatched embryos. Developmental alterations such as circulatory modulation, spinal distortion, and craniofacial deformation were noted at low frequencies (only a few individuals were affected).
Mesripour et al., 2024		Male Swiss mice (9/group)	0.1, 1.5	Oral feeding tube,15 days, 30 days (0.1 mg/kg bw)		Study evaluating the effects of acetamiprid exposure on depressive-like behaviours and grip strength (GS) in mice - The locomotor activity, forced swimming test (FST), and sucrose preference (SP) test After a week ACE dose dependently increased the immobility time during FST, and after 15 days' depressive behaviour was observed equally for ACE (0.1–5 mg/kg). The subacute exposure (0.1 mg/kg) significantly increased the immobility time, SP also declined that revealed anhedonia. These behavioural changes showed that acetamiprid can initiate depressive effects. The changes in locomotor activity were not significant. GS significantly reduced following a week of exposure to acetamiprid (1–5 mg/kg) that indicated neurotoxicity.

Table IX.78: Overview of repeated exposure animal studies

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Chakroun et al. 2016	(Phogat et al., 2022; Zuščíková et al., 2023)	Wistar Rats	1/20, 1/10, and 1/5 of LD50 (10.85, 21.7, 43.4)	Oral, 60 days, acetamiprid		A significant decrease in the body weight gain at the highest dose An increase in the relative liver weight Significant decrease in RBC, HGB, and HCT in rats treated with higher doses of acetamiprid Significant increase in WBC and PLT Induced liver toxicity measured by the increased activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphates (ALPs), and lactate dehydrogenase (LDH) which may be due to the loss of hepatic membrane architecture and hepatocellular damage Significant decrease in the levels of superoxide dismutase and catalase activities
Karaca et al. 2019	(Phogat et al., 2022; Zuščíková et al., 2023)	male Sprague Dawley Rat	12.5, 25 and 35	Oral, 90 days, acetamiprid		Oxidative damage and affected the liver, denoted by injury markers including the levels of cholesterol, and alanine aminotransferase and aspartate aminotransferase enzymes Decrease in plasma urea, uric acid and creatinine levels, all of which might result from liver injury Acetamiprid was more toxic to the liver than the kidney according to the histopathological examinations Acetamiprid exhibited hepatotoxic potential at all treatment doses on
Dhouib et al. 2017	(Phogat et al., 2022; Zuščíková et al., 2023)	Male Wistar rat	40	Oral, 21 days		ACE treatment substantially impairs the survival of primary neuronal cells through the induction of necrosis concomitantly with the generation of an oxidative stress Altered activity of AChE in plasma and cerebellum, and BChE activity in cerebellum Elevated MDA levels, diminished intracellular Ca2+ and activities of SOD and CAT

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Anonymous 1999e **EC 2018** **Hatch 1994**	JMPR 2005 (Anadón et al., 2020; CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	Sprague Dawley rats (60/sex/dose)	0, 160, 400, 1000 ppm (0, 7.1, 17.5, 46.4 mg/kg bw/d in males, 0, 8.8, 22.6, 60.0 mg/kg bw/d in females)	Two-year dietary toxicity with NI-25 (acetamiprid, 99.38%) Oral,	NOAEL: 160 ppm (7.1 and 8.8 mg/kg bw/d for males and females, respectively)	10 rats were euthanized at 12 months Survival was not affected by treatment Mean body weights and food consumption were significantly reduced in males at 1000 ppm and females at 400 ppm Increased mean relative liver weights in HD animals (sign. In males) Lower triglycerides at 1000 ppm females after 12 and 18 months Necropsy did not reveal gross abnormalities related to treatment Combined microscopic revealed liver as target organ Trace to mild centrilobular hepatocellular hypertrophy in males from 400 ppm and in females at 1000 ppm Trace to moderate hepatocellular vacuolation observed in males from 400 and trace to severe micro concretions in the renal papillae in males at 1000 ppm. While decreased body weight and food intake were observed in the high-dose level, no evidence of irreversible neurotoxic effects was observed in the dose range assayed.
Anonymous 1999a **Goldenthal 1994**	JMPR 2005 (CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	Mice (not specified) (60/sex/dose) GLP	0, 130, 400 or 1200 ppm corresponding to 0, 20.3, 65.6, 186.3 mg/kg bw/d in males and 0, 25.2, 75.9, 214.6 mg/kg bw/d in females	18 months dietary study with NI-25 (acetamiprid, 99.38%)	NOAEL: 130 ppm (20.3 and 25.2 mg/kg bw/d for males and females, respectively)	10 mice were sacrificed after 1 year Survival was not affected by treatment Mean bw and mean bw gains statistically significantly reduced at 400 ppm in males Increased liver weights in females at 400 ppm Decreased food consumption in HD males Statistically significant increase in hepatocellular hypertrophy in HD mice at interim sacrifice Incidence of amyloidosis at terminal sacrifice was increased in several organs at the top-dose and in the adrenal cortex and kidney from 400 ppm but within HCD

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Nukui and Ikeyama 1992a	(FAO and WHO, 2012)	Cij: CD-1(ICR) mice (10/sex/dose) GLP	0, 400, 800, 1600, 3200 ppm (0, 53.2, 106.1, 211.1 and 430.4 mg/kg bw/d in males and 64.6, 129.4, 249.1 and 466.3 mg/kg bw/d in females	13 weeks dietary study, acetamiprid (purity 99.2%)	NOAEL: 400 ppm (53.2 and 64.6 mg/kg bw/d in males and females, respectively)	Tremor in HD females with two deaths Two HD males died Decreased bw and food consumption in males and females at 1600 and 3200 ppm No treatment-related ophthalmological effects Significant decrease in haemoglobin in 1600 ppm females, no examination at 3200 ppm due to marked growth depression in both sexes Decreased total cholesterol in females from 800 ppm and in 3200 ppm males, decreased glucose in 1600 ppm males and HD males and females, blood urea significantly increased in HD males and females Urinary pH significantly decreased in HD males Rel . liver weight sign. Increased from 800 ppm in males and females, decreased organ weights being attributed to decreased bw No compound-related lesions at necropsy Centrilobular hepatocellular hypertrophy in HD males and females
Nukui and Ikeyama 1992b	(FAO and WHO, 2012)	Crj:CD(SD) rats (10/sex/dose) GLP	0, 50, 100, 200, 800, 1600 ppm (0, 3.1, 6.0, 12.4, 50.8, 99.9 mg/kg bw/d in males and 0, 3.7, 7.2, 14.6, 56.0 117.1 mg/kg bw/d in females	13 weeks dietary study, acetamiprid (purity >99%)	NOAEL: 200 ppm (12.4 and 14.6 mg/kg bw/d in males and females, respectively)	No increased mortality, no clinical signs Bw and food consumption in males and females from 800 ppm significantly decreased No treatment-related ophthalmological effects No effects on haematological parameters Total cholesterol increased in HD males (significantly) and females Rel. liver weight sign. Increased in males and females from 800 ppm Abs. nd rel. thyroid weight sign. Increased in males from 800 ppm Increased rel testis, brain, lung, heart, kidney weights and decreased abs. adrenal, heart and kidney weights in HD

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						corresponding to decreased bw and without histopathological correlates No compound-related lesions at necropsy Dose related centrilobular hepatocellular hypertrophy in males and females from 800 ppm
Auletta 1992	(FAO and WHO, 2012)	Beagle dogs (2/sex/dose) GLP	250, 500, 1000, 3000 ppm (8-9, 16-18, 22-34, 21-81 mg/kg bw/d in males and 6-10, 15-22, 28-43 and 23-82 mg/kg bw/d in females) 3000 ppm group started with 125 ppm which was increased at day 12 after lack of significant toxicity at 1000 ppm	28-day dietary study, acetamiprid (purity 99.46%)	NOAEL: 1000 ppm (22-34 and 28-43 mg/kg bw/d in males and females, respectively)	No increased mortality Marks bw loss and decreased food consumption with emaciated appearance in 3000 ppm animals, only slight effects at 1000 ppm No effects on haematology and clinical chemistry Organ weights comparable to control or were consistent with bw loss
Auletta 1994a	(FAO and WHO, 2012)	Beagle dogs (4/sex/dose) GLP	0, 320, 800, 2000 ppm (0, 13, 32, 58 mg/kg bw/d in males and 0, 14, 32, 64 mg/kg bw/d in females)	3-months dietary study, acetamiprid (purity 99.36%)	NOAEL: 800 ppm (32 mg/kg bw/d for males and females)	No increased mortality Bw and food consumption decrease during first 2 weeks in HD animals, very little decrease in MD Clinical observations and ophthalmological examinations without effect No clear effects in haematology, biochemistry and urinalysis Thyroid/parathyroid weights dose-related decreased in all treated females only (toxicological significance not clear in absence of morphological alterations)

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Auletta 1994b	(FAO and WHO, 2012)	Beagle dogs (4/sex/dose) GLP	0, 240, 600, 1500 ppm (0, 9, 20 and 55 mg/kg bw/d in males and 0, 9, 21 and 61 mg/kg bw/d in females)	12-months dietary study, acetamiprid (purity >99%)	NOAEL: 600 ppm (20 and 21 mg/kg bw/d for males and females, respectively)	No increased mortality Clinical observations and ophthalmological examinations without effect Moderate bw loss in HD females during week 1 and 2 and HF males between week 2 and 3 remained lower until study termination No clear effects in haematology, biochemistry and urinalysis No adverse effects on organ weights No compound-related lesions at necropsy No compound-related microscopic changes
Trutter 1997	(FAO and WHO, 2012)	New Zealand White rabbits (Hra:[NZW] SPF) (5/sex/dose) GLP	0, 100, 500, 1000	3-week dermal irritation and systemic toxicity study (5 days/week) acetamiprid (purity 99.9%)	NOAEL: 1000	No increased mortality No adverse effects on clinical observations, body weight and food consumption No signs of compound-induced dermal irritation No adverse effects on haematology, serum chemistry, necropsy or organ weights No compound-related histomorphological findings in the tissues examined (liver, kidney, skin)
Ivett 1999	(FAO and WHO, 2012)	Sprague-Dawley Crl:CD BR rats (10/sex/dose) (GLP)	0, 200, 600, 1800, 5400 ppm (0, 12.8, 36.5, 112.2, 319.3 mg/kg bw/d for males and 0, 15.6, 44.6, 135.6, 356.1 mg/kg bw/d for females, respectively)	Dietary, 13 weeks, Metabolite IM-1-4	NOAEL: 600 ppm (36.5 mg/kg bw/d) for males and 1800 ppm (135.6 mg/kg bw/d) for females	No increased mortality No clinical or macroscopic pathology findings No effects on ophthalmology, haematology and Coagulation, urinalysis or organ weights Significant compound effects on mean feed consumption and body weights that were likely related to reduced diet palatability in HD Increased pigment in the splenic sinusoids in males from 1800 ppm and in HD females

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Nukui and Ikeyama 1997	(FAO and WHO, 2012)	Crj:CD(SD) Rats (10/sex/dose) (GLP)	0, 160, 800, 4000, 20 000 ppm (0, 9.9, 48.9, 250.1, 1246.6 mg/kg bw/d in males and 0, 11.1, 55.9, 275.9, 1173.7 mg/kg bw/d in Females)	Dietary (purity 98.94%), 13 weeks, metabolite IM-O	NOAEL: 800 ppm (48.9 mg/kg bw/d) for males and 4000 ppm (275.9 mg/kg bw/d) for females	No increased mortality or clinical signs Decreased bw, bw gain and food consumption in HD males and females No ophthalmological effects No effects on haematology Sign. increased serum AP activity in HD females No effects on urinalysis Sign. decreased abs. lung and liver weights in HD males, sign. increases in rel. brain weights in HD males and females, lung and liver weights in HD females, and kidney weights (both sexes) No compound-related lesions at necropsy Dose-related eosinophilic intranuclear inclusions were seen in the proximal Tubular epithelium of kidneys for 20 000 ppm males and females and 4000 ppm males
(Dukhnytskyi et al., 2021)		Male mice (not specified) (7dose)	0, 65	Mospilan RP given orally via drinking water for 30 days		Clinical signs, food and water consumption were normal Treated animals were more aggressive, and often attacked each other WBC and platelet count increased Leucocytosis had a neutrophilic character with a shift of the neutrophil nucleus to the right leading to an increased number of segment-nuclear neutrophils to 1.5 times Total protein + globulin sign. Increased, urea, sign, decreased ALAT increased

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
(El-Bialy et al., 2020)		Male albino rats (not specified) (8/group)	0, 2.14	Gavage, 45 days		A mixture from acetamiprid and cyhalothrin Increased ALT, AST, decreased total protein and globulin In liver, increased vacuolar degeneration, hepatocellular necrosis, sinusoidal monocytosis and portal hepatitis Necrosis of some renal tubular epithelium with nuclear pyknosis and mild intertubular mononuclear cell infiltration Most of the effects could be mitigated by simultaneous administration of ginseng
(EL-Hak et al., 2022)		Male Wistar rats (8/group)	0, 5, 10, 20	Gavage, 60 days	5	Dose dependent decrease in serum calcium and total protein and in serum oxidative/antioxidant biomarker levels Mononuclear cell invasion, hydropic degeneration, and congestion of the central vein and hepatic artery
Mondal et al. 2014	(Phogat et al., 2022)	Female Wistar Rats	25, 100, 200	Oral, 28 days, acetamiprid		Fibrosis, granularity of cytoplasm and fatty changes were observed in dose-dependent manner in liver tissue
Devan et al. 2015	(Phogat et al., 2022)	Wistar Rats	27.5, 55, 110	Oral, 90 days, acetamiprid	≤55	Elevated AST, and ALT levels, and declined brain AChE activity Elevated plasma cholesterol Decreased food intake, body and organ weight Reduced CAT, SOD, and GPx activities and GSH levels Increased LPO and lesions in liver All changes were observed at intermediate and high dose in hepatic and renal tissues
Doltade et al. 2019	(Phogat et al., 2022)	Male Wistar Rats	26.25, 105	Oral, 28 days, acetamiprid		Increased LPO Decreased GSH content, GR, CAT and SOD activities and caused histological alterations in liver and kidney tissue
Shahin 2018	(Phogat et al., 2022)	Male albino rats	100	Oral, 30 days, acetamiprid		Altered serum lipid profile, liver function profile and cytokinine levels Increased TBARS content and decreased GSH content and GPx activity in liver tissue

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
El-Bialy et al. 2020	(Phogat et al., 2022)	Male Wistar Rats	2.14	Oral, 45 days, commercial formulation of acetamiprid and lambda-cyhalothrin		Elevated ALT and AST activities, urea and creatinine concentration Declined total protein and albumin levels in serum Increased MDA levels, nitric oxide, caspases-3 expression Decreased GSH content and CAT activity, and damaged hepatic and renal tissues
Yan et al. 2020	(Phogat et al., 2022)	Mice	0.5	Oral, 30 days, acetamiprid, nitromethyl, dinotefuran individually		Altered body and organ weight Decreased GSH content, increased MDA levels, SOD activity Disturbed fatty and amino acids metabolism and increased triglycerides and total cholesterol in liver
Alhusaini et al. 2019	(Phogat et al., 2022)	Male Wistar Rats	100	Oral, 30 days, acetamiprid		Increased levels of urea, uric acid, creatinine and cystatin C Up regulated mRNA expression of NF-kB and TNF- α and also increased ICAM-1 protein expression in serum while down regulated Nrf-2 expressions and IL-10 levels. Depleted GSH content and elevated nitric oxide and MDA levels in kidney tissue
(Erdemli et al., 2020)	(Phogat et al., 2022)	Malb-c mice	0, 25	Gavage, 21 days	LOAEL: 25	Study to investigate protective effects of melatonin and vitamin E on acetamiprid-induced nephrotoxicity MDA and TOS levels significantly increased and GSH, TAS, SOD, and CAT levels decreased TNF-α, IL-6, and IL-1β levels were significantly increased Mild to moderate epithelial damage prevalent in the proximal and distal tubules of kidneys Tubular degeneration and different levels of inflammatory cell infiltration observed in renal parenchyma Extensive congestion in peritubular vascular structures Significant capillary collapse in the glomeruli Significantly less adverse effects when additionally exposed to vitamin E and/or melatonin

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Department of Toxicology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran et al., 2021)		adult male Wistar rats (6/group)	0, 20, 40	Gavage, 28 days		Study to assess the protective effect of quercetin on acetamiprid-induced hepatotoxicity Acetamiprid especially in high dose (40mg/kg), induced hepatic tissue destruction associated with increased hepatic enzyme activity, except ALP (alkaline phosphatase) activity, in the serum. Increased the lipid peroxidation and decreased total thiol content and SOD (superoxide Dismutase) activity, which indicates acetamiprid induced oxidative stress in hepatic tissues Significantly increased ALT (Alanine amino transferase), AST (aspartate amino transferase) and LDH (lactate dehydrogenase) activity in both groups compared with the control. The increase in AST and LDH activity was dose dependent. MDA (Malondialdehyde) level was significantly elevated in the high-dose group compared with the control. Both tested groups - significant decrease was observed in the amount of liver total thiol in comparison with the control. A significant reduction in the SOD activity was observed in the high-dose group Severe liver injuries as pyknosis, vacuolation of hepatocytes and congestion are observed in the tissue section of the rats in high-dose group.
(Li et al., 2021)		C57BL/6 male mice (8/group)	0.5	Gavage, 30 days, acetamiprid		Morphological damage in the liver Treatment with acetamiprid displayed clear vacuolation and infiltration of inflammatory immune cells causing significantly elevated histopathological Suzuki score Also observed margination of leukocytes from the central axial stream to the periphery of vessels and adhesion with lining endothelial cells; slight congestion of central vein and hepatic sinusoids

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						The concentration of AST and ALT were significantly increased Significantly increased the Orai1 and Stim1 expression in the liver Inhibition of Serca (as an essential transporter of calcium ions from the cytoplasm)
(Nur et al., 2022)		Mus musculus var. Albinos male mice	0, 5, 10, 15 (10/group)	Oral gavage, 14 days, acetamiprid		Study investigating the effect of acetamiprid on bcl-2 and p53 immunoreactivity The histopathological examinations revealed the degeneration in the distal and proximal tubules, glomerular degeneration, increase in the capsular area, glomerular atrophy, and haemorrhage were determined in the dose dependent manner Bcl-2 and p53 immunoreactivity was observed in glomerular cells, sinusoidal epithelium, and proximal and distal tubule cells
Didenko et al., 2022		Wistar Han rats	6, 12, 60 (10/group)	Oral, 13 weeks, acetamiprid	NOAEL - 12 (NOEL – 6)	Study to determine the dose dependence of the subchronic effect of acetamiprid on the body of rats based on the data of morphological studies of internal organs At a dose of 60 mg/kg indicated hepatotoxic and nephrotoxic effects, as well as neurotoxic effects with signs of irreversible neurocyte damage.
EL-Gendy et al., 2022		Adult male mice Mus (musculus, BALB/c) (5/group)<	14.143	Oral, 14 days, acetamiprid (97.34%)		Study to investigate haematotoxicity, oxidative stress, hepatotoxicity, nephrotoxicity, immunotoxicity, and histological alterations of acetamiprid, as well as the role of quercetin (QE) in alleviating these effects Significantly lowered the body weight gain, haematological indices, glutathione (GSH), and both cellular and humoral immunity, Lipid peroxidation (LPO), glutathione peroxidase (GPx), and liver and kidney marker values were considerably increased in male mice exposed to ACP. In addition, examination under light microscopic showed that ACP

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						induces histological alterations in liver and kidney tissues.
El-Nagdy et al., 2024		Adult albino rats (10/group)	10	Oral, 12 weeks, acetamiprid (97%)		The study investigating the role of fenugreek seed extract in alleviating pancreatic toxic effects and altering glucose homeostasis induced by acetamiprid via modulation of oxidative stress, apoptosis, and autophagy. Significant elevations in plasma glucose, glycosylated haemoglobin levels (HbA1c), serum amylase, and serum lipase, along with a decrease in plasma insulin levels. Significant increases in tumour necrosis factor-alpha (TNF-α) and malondialdehyde (MDA) were associated with reductions in the levels of interleukin 10 (IL-10), glutathione peroxidase, and catalase. Glucose-6-phosphatase and glycogen phosphorylase were significantly increased, with a significant reduction in hexokinase and liver glycogen stores. These biochemical changes were associated with histological changes in pancreatic sections stained by haematoxylin and eosin, Masson stain, and Orcein stain.
Phogat et al., 2023		Male Wistar rats (6/group)	21.7	Intragastrical, 21 days, acetamiprid		Study evaluating the ameliorative potential of berberine (BBR)—a polyphenolic alkaloid- on acetamiprid-induced oxidative stress, mitochondrial dysfunctioning, and structural changes in different rat brain regions. Induced oxidative stress The AChE activity increased Significantly elevated LPO levels in all three brain regions by 108%, 61%, and 74%, respectively Acetamiprid exposure depleted the GSH content in different regions of the brain. Significantly elicited oxidative stress as evidenced by lipid peroxidation, protein oxidation, and depletion of endogenous antioxidants.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						Elevated NF-κB, TNF-α, IL-1β, IL-6, and IL-12 expression and caused structural alterations in liver tissue. Reduced the activity of the SOD enzyme by 50%, 38%, and 39% in the forebrain, midbrain, and hindbrain regions, respectively
Zeinab Mohamed et al., 2023		Male albinos rats (6/group)	20	Oral, 20/40 days		Study investigating the protective role of curcumin (100 mg/kg body weight) on the neurotoxicity caused by an environmental contaminant, acetamiprid The considerable reduction in the levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and Acetylcholinesterase (AChE) in the brain cortex, as well as the reduction in dopamine and serotonin neurotransmitters in the hippocampus, indicating that acetamiprid has an overall pro-oxidant effect

Table 79: Overview of eye and skin irritation and sensitisation studies

Reference	Cited in	Findings
Mochizuki & Goto 1993a	(FAO and WHO, 2012)	Not irritant in a primary dermal irritation test in male New Zealand White rabbits
Trutter 1997	(FAO and WHO, 2012)	No dermal irritation in New Zealand White rabbits, dermally treated with up to 1000 mg/kg bw/d for three weeks (5 days/week)
Mochizuki 1994a	(FAO and WHO, 2012)	No sensitising potential in a guinea pig maximization test (GPMT) with female Dunkin/Hartley guinea pigs
Coleman 1997	(FAO and WHO, 2012)	No sensitising potential in a delayed contact hypersensitivity test with male and female Hartley guinea pigs
Mochizuki & Goto 1993b	(FAO and WHO, 2012)	Not irritant in an eye irritation study in male New Zealand White rabbits

Table IX.80: Overview of genotoxicity studies

Reference	Cited in	Findings
Cifone 1994	(FAO and WHO, 2012)	In vitro mouse lymphoma forward mutation assay in L5178Y Tk+/− cells (TK locus) with 63.5–2000 μg/mL negative without metabolic activation but questionable results with metabolic activation
Kanaguchi 1992b	(FAO and WHO, 2012)	In vitro DNA repair test on bacteria (rec assay) in *Bacillus subtilis* M45(rec−) and H17(rec+) at 1359, 2718, 5435, 10 870, 21 740 μg/disc without metabolic activation, 679.4, 359, 2718, 5435, 10 870 μg/disc with metabolic activation negative
Kanaguchi 1997b **Kanaguchi 1993a**	(CLH report, 2018; FAO and WHO, 2012)	In vitro Ames test negative with and without metabolic activation in TA98, TA 100, TA 1535, TA 1537 E.coli WP2 uvrA at 313, 625, 1250, 2500, 5000 μg/plate
Adams 1998 **Adams 1997**	(CLH report, 2018; FAO and WHO, 2012)	In vitro HGPRT gene mutation in Chinese hamster ovary (CHO) cells negative with and without metabolic activation at up to 4000 μg/mL with and without metabolic activation
Kanaguchi 1997a **Kanaguchi 1992a**	(CLH report, 2018; FAO and WHO, 2012)	In vitro Chromosome aberration in CHO cells at concentrations of 17.0, 33.9, 67.8, 135.6, 271.3, 542.5, 1085, 2170 μg/mL positive with metabolic activation, weakly positive without metabolic activation in combination with medium cytotoxicity
Ham 1998 **Ham 1994**	(CLH report, 2018; FAO and WHO, 2012)	In vitro Unscheduled DNA synthesis in primary rat hepatocytes negative at 10.1 – 505 μg/mL
Senyildiz et al. 2018	(Zuščíková et al., 2023)	In vitro Comet assay positive with DNA damage in human neuroblastoma cells (SHSY-5Y) and human hepatocellular carcinoma cells (HepG2) after ACE treatment in a dose-dependent manner
Annabi et al. 2019	(Zuščíková et al., 2023)	In vitro Comet assay positive in a dose dependent manner with a significantly negative impact on cell viability and apoptosis (caspase-dependent) in PC12 cells treated with ACE (100–700 M)
Anonymous 1998d **Muri 1994a**	(CLH report, 2018; FAO and WHO, 2012)	In vivo micronucleus test in CD-1 mice (10/sex/dose) at 20, 40, 80 mg/kg bw by a single gavage application negative
Anonymous 1998e **Durward 1993**	(CLH report, 2018; FAO and WHO, 2012)	In vivo Chromosomal aberration test in bone marrow of SD rats (5/sex/dose) after a single oral dose of 250 mg/kg bw via gavage negative
Anonymous 1997i **San and Sly 1997**	(CLH report, 2018; FAO and WHO, 2012)	In vivo unscheduled DNA synthesis in liver cells of SD rats (3 males/dose) at 75, 150, 300 mg/kg bw negative
Kanaguchi 1997	(FAO and WHO, 2012)	Metabolite IM-1-5: Ames test in S. typhimurium TA98, TA100, TA1535, TA1537 up to a concentration 5000 μg/plate negative with and without metabolic activation
Mochizuki and Kanaguchi 1994c	(FAO and WHO, 2012)	Metabolite IM-1-2: Ames test in S. typhimurium TA98, TA100, TA1535, TA1537, E.coli WP2uvrA up to a concentration of 5000μg/plate negative with and without metabolic activation

Reference	Cited in	Findings
Kanaguchi 1999b	(FAO and WHO, 2012)	Metabolite IB1-1: Ames test in S. typhimurium TA98, TA100, TA1535, TA1537 up to a concentration of 5000 µg/plate negative with and without metabolic activation
(H.-X. Zhang et al., 2023)		Investigated effects of acetamiprid (100 µL acetamiprid for 72 h) on cell viability, reactive oxygen species (ROS) levels, DNA damage, cellular oxidized nucleotide concentrations, and oxidative damage to tRNA in HepG2 cells and LO2 cells Several tRNA fragments, CUC[m1A]Gp, CACGp, [Cm]C[m2G]p, and DDGp, are significantly downregulated in acetamiprid-treated HepG2 cells, while only [Cm]C[m2G]p in acetamiprid-treated LO2 cells Cytotoxicity - IC50 values were 5.95 ± 0.32 mM and 7.31 ± 0.25 mM for HepG2 and LO2 cells, respectively Acetamiprid increased ROS production in a dose dependent manner in either HepG2 or LO2 cells (After exposure to 2 mM acetamiprid for 24 h, the rates of ROS generation were approximately 4.7 and 0.35 times higher in HepG2 and LO2 cells than in corresponding untreated groups) Acetamiprid induced the significant increment of γ-H2AX expression over a range of 1–4 mM in either HepG2 or LO2 cells, compared with pretreatment levels Acetamiprid caused the increased ROS levels, significant DNA damage, upregulated oxidized nucleotide concentrations, and accumulated 8-oxo-G in tRNA
(Sandayuk and Aksu Kiliçle, 2020)		Study aimed to determine the genotoxic effect of Acetamiprid in mus musculus var. albinos bone marrow cells by chromosomal aberration (CA) and micronucleus (MN) analysis Mice were exposed to 5, 10, and 15 mg/kg Acetamiprid orally for 14 days No genotoxic effects were observed in low and medium dose group; CA rates at high dose group were determined to be high; MI ratio at the high dose group was lower than the control group. The ratios of the polychromatic erythrocyte with micronucleus - No significant differences in low and medium dose group, however statistically significant increase in MNPCE rates observed in the high dose group. In the study concluded that acetamiprid at 15 mg/kg dose was genotoxic-cytotoxic.
(Baysal and Atlı-Eklioğlu, 2021)		Human colorectal adenocarcinoma cell line HT-29 Concentration: 1–4000 µM Induced cytotoxicity by caspase-mediated apoptosis, mitochondrial membrane depolarization, DNA damage, and oxidative stress

Table IX.81: Overview of carcinogenicity studies

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration	Findings
Anonymous 1999e **Hatch 1994**	JMPR 2005 (CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	Sprague Dawley rats (60/sex/dose)	0, 160, 400, 1000 ppm (0, 7.1, 17.5, 46.4 mg/kg bw/d in males, 0, 8.8, 22.6, 60.0 mg/kg bw/d in females)	Two-year dietary study with NI-25 (acetamiprid, 99.38%)	10 rats were euthanized at 12 months Survival was not affected by treatment Mean body weights significantly reduced in males at 1000 ppm and females at 400 ppm Significant trend increase of mammary gland adenocarcinoma was depending on statistical test and was lying within HCD, therefore considered not treatment related Hyperplasia in the mammary gland was increased significantly in HD females (62%) which was slightly outside of the range of HCD (0-58.6%)
Anonymous 1999a **Goldenthal 1994**	JMPR 2005 (CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	Mice (not specified) (60/sex/dose)	0, 130, 400 or 1200 ppm corresponding to 0, 20.3, 65.6, 186.3 mg/kg bw/d in males and 0, 25.2, 75.9, 214.6 mg/kg bw/d in females	18 months dietary study with NI-25 (acetamiprid, 99.38%)	10 mice were sacrificed after 1 year Survival was not affected by treatment Statistically significant increase in myeloid hyperplasia of the femoral bone marrow in HD males was considered not treatment related Incidence of amyloidosis in the terminal sacrifice was increased in several organs at the top-dose and in the adrenal cortex and kidney from 400 ppm but within HCD No neoplasms at a statistically significantly increased incidence noted at any dose
(X. Li et al., 2022)		Female BABL/c mice	1, 10, 30 (5/group)	3 weeks, Gavage	Study focused on investigation breast cancer progression via real-time observation by bioluminescence imaging. 4T1-Luc (4T1 cells labeled with luciferase) cell model was used in *in silico*, *in vitro* and *in vivo* study. In vitro - acetamiprid concentration: 10 nM, 100 nM, 1 μM, 10 μM and 50 μM; investigated the activity of acetamiprid on GPER signal pathway by calcium mobilization assay; Results - agonistic activity with the lowest observed effective concentration (LOEC) of 1 μM In silico - acetamiprid with GPER activity induced 4T1 breast cancer cells migration and showed the highest potency with LOEC of 100 nM., also induced 4T1 cells proliferation at high concentration of 50 μM and up-regulated GPER expression in a dose-dependent manner. In vivo - using 4T1-Luc cells injected orthotopic tumour model. It was found that acetamiprid promoted in-situ breast cancer growth.

Table IX.82: Overview of fertility studies

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Arican et al. 2020	(Phogat et al., 2022; Zuščíková et al., 2023)	Male Rat	12.5, 25 and 35 mg/kg	Oral, 90 days, acetamiprid	-	Plasma testosterone levels and sperm count both decreased in a dose-dependent manner. Gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH) levels increased at low and medium dose groups and acetamiprid caused lipid peroxidation and glutathione (GSH) depletion in the testes Acetamiprid induced apoptosis in medium and high dose groups and proliferation index dramatically decreased in high dose group Toxicity on male reproductive system in the high dose. The mechanism of the toxic effect may be associated with oxidative stress, hormonal disruptions and apoptosis.
Ibrahim et al. 2020	(Phogat et al., 2022; Zuščíková et al., 2023)	Male Rat (6/group)	¼ and ½ of LD50 (55, 110)	Oral, 28 days, acetamiprid (98%)	-	Inhibited the synthesis of adenosine triphosphate (ATP) and cyclic adenosine monophosphate (cAMP) in Leydig cells Induce oxidative stress leading to decrease antioxidant enzymes and damage Sertoli cells resulting in decreasing testosterone level and effect on fertility The histological investigation revealed that exposure to acetamiprid disorganized stratification of Spermatogonia, primary and secondary spermatocytes in some tubules shedding of sperm cells into lumina, necrosis in tubules and reduction of the primary and secondary spermatids
Kenfack et al. 2018	(Phogat et al., 2022; Zuščíková et al., 2023)	Male Guinea pig	26.67, 40 and 80	Oral, 90 days, acetamiprid	-	Induced a reduction in the weight of the testes, epididymides, vas deferens, and supporting glands. The testicular structure, testosterone concentration, testosterone response time, and epididymal sperm characteristics were also negatively affected
Zhang et al. 2011	(Phogat et al., 2022; Zuščíková et al., 2023)	Kunmin male mice	30	Oral, 35 days, acetamiprid	-	Increasing concentrations of reactive oxygen species (ROS) in the testes, decreasing the overall weight of testosterone-responsive organs, the concentration of serum testosterone, and sperm quality

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						Decreased sperm count, sperm motility and viability, CAT, GPx and SOD activities Increased MDA and nitric oxide concentration and anti-phosphor p38 expression Caused histological alterations
(Toghan et al., 2022)	(Zuščíková et al., 2023)	Albino rat (7/group)	10	Gavage, 5 weeks	-	Decreasing in the motility, count, and viability of sperm as well as reductions in serum testosterone and gonadotropin-releasing hormone levels
Kong et al. 2017	(Phogat et al., 2022; Zuščíková et al., 2023)	male Sprague Dawley rats	10, 30	Oral, 35 days, acetamiprid	-	Decreases in the numbers of spermatids, epididymal sperm, and Leydig cells, together with decreases in the expression levels of Star (steroidogenic acute regulatory protein), Cyp11a1, Hsd3b (3-β-hydroxysteroid dehydrogenase), and testis weight Changes in the morphology of seminiferous tubules
(Zayman et al., 2022)	(Dehdari Ebrahimi et al., 2023)	Mice	25	Gastric gavage, 21 days		Decreased seminiferous tubule diameter and epithelial thickness, epithelial degeneration, decreased spermatozoa in the lumen, decreased PAS-positive staining in the seminiferous epithelial basement membrane, oedema in the interstitial area, and hydropic degeneration in Leydig cells Degeneration in tubule cells, lysosomal accumulation in cells of the spermatogenic line, vacuolisations with myelin figures, and necrosis Hydropic degeneration, electron-dense lipid vacuoles, and chromatolysis were evident in the Leydig cell cytoplasm. In Sertoli cells, electron-dense lysosomal deposits were noted. In biochemical terms, there were decreased tissue glutathione (GSH) and total antioxidant status (TAS), and increased malondialdehyde (MDA) and total oxidant status (TOS). Plasma luteinizing hormone (LH), follicular stimulating hormone (FSH), and testosterone levels were decreased
Schardein 1991	(FAO and WHO, 2012)	Crl:CD VAF/Plus rats (5/sex/group), range-finding study for	0, 125, 250, 500, 1000 ppm (0, 8.3, 16.6, 31.4, 59.7 mg/kg bw/d for males	Dietary (99.38% purity), 14 days prior to mating and continuing until euthanasia	NOAEL: 250 ppm (16.6 and 18.0 mg/kg bw/d for males and	No clinical signs of toxicity or gross necropsy findings were observed for any animal Treatment-related decreased bw and food consumption at 500 and 1000 ppm

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
		multigeneration study (GLP)	and 0, 9.0, 18.0, 33.7, 63.6 mg/kg bw/d for females)		females, respectively)	No indication of impaired fertility Offspring growth was inhibited (reduced litter weights) by treatment of F0 dams with 500 and 1000 ppm Development of F1 offspring also adversely affected (decreased number of implantation sites) at 1000 ppm
Anonymous 1999d **York 1994** **Trutter 1999**	(CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	Crl:CD VAF/Plus rats (26/sex/group), 2-Generation study (OECD 416, GLP)	0, 100, 280, 800 ppm (0, 6.5/7.5, 17.9/21.0, 51.0/63.3 mg/kg bw/d for F0/F1 males, 0, 7.6/8.4, 21.7/23.8, 60.1/72.6 mg/kg bw/d for F0/F1 females during premating, 0, 6.8/6.6, 18.5/18.5, 50.9/55.2 mg/kg bw/d for F0/F1 during gestation and 13.2/13.7, 37.5/40.3, 108.1/105.5 mg/kg bw/d for F0/F1 during Lactation	Dietary (99.38% purity), at least 10 weeks prior to mating, throughout mating, gestation and lactation periods, terminated after weaning of F2	NOAEL for systemic adult toxicity: 100 ppm (6.5 and 7.6 mg/kg bw/d for males and females, respectively) NOAEL for offspring toxicity: 280 ppm (21.0 and 23.8 mg/kg bw/d for males and females, respectively) NOAEL for reproductive toxicity: 800 ppm	F0 parents: no treatment related deaths, lower bw at 800 ppm throughout the study (< than 8% relative to ctrl during premating for both sexes), significantly decreased food consumption (20%) at HD females from GD0-20 No compound-related histopathological effects, decreased relative kidney weights, no changes in reproductive organs, epididymides of HD males autolysed due to an error and where not investigated, in a then conducted supplemental study coincident with 26 males fed to 800 ppm or control showed no compound related effects on total testicular and epididymal sperm counts or on sperm morphology F1 parents: two LD and five HD females experienced total litter deaths, no deaths of adults Decreased bw and food consumption in HD animals during all phases No compound related histopathological changes and no effects on reproductive organs or functions No reproductive effects on mating performance, fertility, reproduction parameters, oestrus cycle for F0 and F1 adults F1 pups: viability and weaning indices unaffected In HD mean number of live pups/litters very small but significant decrease on day 14 and 21 with questionable biological significance Pup bw decreased in utero and growth significantly decreased throughout lactation (11-24%) No anogenital distance recorded for F1 pups, preputial separation and vaginal opening significantly increased in correlation with decreased bw

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						No treatment related changes during necropsy in F0 adults or F1 pups F2 pups: viability and weaning indices significantly affected in HD animals, pup mortality greatly increased from PND0-21, slightly decreased bw but strongly decreased post-natal growth (15-22%) Anogenital distance not affected, no data on preputial separation and vaginal opening because F2 pups were sacrificed on LD21 Sign. decreased in-utero growth, post-natal growth and bw during lactation in HD pups Mean age for reaching eye opening in HD pups significantly increased, correlating to decreased bw, no necropsy changes in F1 adults and F2 pups No effects on reproductive performance or fertility
Anonymous 1997f	(CLH report, 2018; RAC Opinion, 2020)	Rats, 90-day oral toxicity study	0, 800, 1600 ppm	Oral, 90 days		Increased rel testis weight (+13%/+19% at 800/1600 ppm), not significant Decreased bw -14%/-13% at 800/1600 ppm), significant
Anonymous 1992	(CLH report, 2018; RAC Opinion, 2020)	Mouse, 90-day oral toxicity study		Oral, 90 days		Increased rel. testis weight (+37% at 3200 ppm), significant Decreased abs. and rel. ovary weight (-65%/-52% at 3200 ppm / 466 mg/kg bw/day), significant
Anonymous 1999a	(CLH report, 2018; RAC Opinion, 2020)	Mice (60/sex/dose), 18-month carc study	0, 130, 400 or 1200 ppm corresponding to 0, 20.3, 65.6, 186.3 mg/kg bw/d in males and 0, 25.2, 75.9, 214.6 mg/kg bw/d in females	Diet, 19 months		Decreased abs. prostate weight in interim and terminal sacrifice groups (-39%/-23% at 1200 ppm / 186 mg/kg bw/day), significant

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Zhang et al. 2011	(RAC Opinion, 2020)	Kunmin mal mice (10/dose), 35 day-tox-study	0, 30…….?	Gavage, 35 days	LOAEL: 30 mg/kg bw/d	Decreased bw gain (-38%) and rel. testis and epididymides weight (-17%), seminal vesicles and prostate gland (-17%) Decreased serum testosterone level (Vitamin E reduced these effects) Sign. decreased sperm number (-76%), viability (13%) and motility (52%) with sign. Increased rate of acrosome deformity (Vitamin E reduced these effects) Vacuolization of the seminiferous tubules and number of spermatids and interstitial Leydig cells decreased, degeneration of the mitochondria and endoplasmic reticulum within the Leydig cells RAC concluded effects were due to different impurities and were not representative for the form of acetamiprid marketed in Europe
Arıcan et al., 2020		SD rats	0, 12.5, 25, 35	Gavage, 28 days	NOAEL: 12.5	Bw non-significantly decreased in alle treated groups Liver steatosis and slowness of movements in HD Testes weights decreased similar to bw in all treatment groups Sperm number significantly decreased in MD and HD The ratio of normal to abnormal sperms did not differ between the groups Significantly increase in flattened headed sperms was observed in HD Plasma testosterone (≥ 23%) and cholesterol (≥ 12%) levels decreased in a dose dependent manner Histopathology of testes showed sign. Decreased seminiferous tubule score and proliferation index in all treatment groups Apoptotic index was sign. Increased in MD and HD According to authors, the toxic effect may be associated with oxidative stress, hormonal disruptions and apoptosis.

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
EL-Hak et al., 2022		Male Wistar rats (8/group)	0, 5, 10, 20	Gavage, 60 days		Sperm count and motility significantly and dose dependently decreased LH land FSH levels were significantly lower
Chawseen 2011	(Phogat et al., 2022)	Male mice	0.16, 0.22 mL/L acetamiprid and 3.125, 4.166 mg/L glyphosate, drinking water	Oral, 28 days		Decreased serum testosterone level, increased thickness and diameter of seminiferous tubules; induced lumen dilation and degeneration in mice testis tissue in dose-dependent manner Both pesticides showed synergistic effects in combination
Mosbah et al. 2018	(Phogat et al., 2022)	Male Wistar Rats	27	Oral, 45 days, acetamiprid		Decreased plasma testosterone level and elevated TBARS levels, declined body and reproductive organ weight gain, spermatids number, sperm count, sperm viability Induced sperm abnormality
Hassanzadeh et al., 2023		NMRI female Mice (6 in each group)	0, 10, 20	intraperitoneal injections, 1 month, acetamiprid		Study examined the protective effect of melatonin against toxic pathologic changes from acetamiprid on reproductive system parameters of female mice (ovarian histopathological changes and oocyte quality based on in vitro fertilization (IVF) parameters) Histopathology showed abnormalities as compared to controls. Inflammation was the most common change seen in the ovarian tissue of mice from all groups. Inflammatory cells were seen in both ovarian stroma and follicular fluid in the follicles. Androgen-secreting cells and vascular hyperaemia were present in the medulla area of the ovaries and peripheral area of the follicles. The number of Graafian follicles in the groups given low and high doses of acetamiprid was significantly lower compared to mice given saline (control). The number of mature oocytes in the mice in both doses was significantly lower than in mice given saline Induced histological damages in the ovaries of mice and caused increases in the number of atretic follicles and decreases in the quality of oocytes based on IVF parameters.

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Salehi Mishani et al., 2022		BALB/c mice (70 male and 35 female) (5 males/group)	1.5 ppm, 2.5 ppm (in drinking water)	Oral (in drinking water), 60 days, acetamiprid		Evaluation of the fertility of male mice which were treated with acetamiprid and after were mated with a group of 35 adult females. Significantly reduced the body weight, food consumption, testosterone production, testicular germ cells and embryo numbers at high dose. Increment in the levels of follicle-stimulating hormone and luteinizing hormone in mice at high dose. Study of very low quality.
Sevim et al., 2023		Wistar Albino male rats	12.4	Oral, 90 days, acetamiprid		Study investigated the positive effects of probiotic Saccharomyces boulardii on the male reproductive system under low dose neonicotinoid pesticide exposure conditions. Within histopathological findings the exposure to acetamiprid caused severe degeneration and necrosis which has been detected/observed in spermatocytes at tubular wall; severe oedema detected at intertubular region and hyperaemia detected in blood vessels. Severe intracytoplasmic 8-OHdG and Caspase-3 expressions were detected. Sperm count and morphology – no significant differences

Table IX.83: Overview of studies developmental toxicity studies

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Anonymous 1999d **York 1994** **Trutter 1999**	(CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	SD rats, 2-Generation study (OECD 416, GLP)	0, 100, 280, 800 ppm (0, 6.5/7.5, 17.9/21.0, 51.0/63.3 mg/kg bw/d for F0/F1 males, 0, 7.6/8.4, 21.7/23.8, 60.1/72.6 mg/kg bw/d for F0/F1 females during premating, 0, 6.8/6.6, 18.5/18.5, 50.9/55.2 mg/kg bw/d for F0/F1 during gestation and 13.2/13.7, 37.5/40.3, 108.1/105.5 mg/kg bw/d for F0/F1 during Lactation	Dietary, at least 10 weeks prior to mating, throughout mating, gestation and lactation periods, terminated after weaning of F2	NOAEL: 280 ppm	Evidence of limited maternal toxicity in F0 HD females with decreased bw (8-10%), bw gain (8%) and food consumption (20%, significant) and in F1 HD females with decreased bw (12-14%, significant), bw gain (8%), food consumption (13%, significant) F1 HD pup body weights decreased in utero (7%, significant) and postnatal (11-24%, significant), similar in F2 Viability and weaning indices clearly effected in HD No effect on anogenital distance, but only recorded in F2 pups Preputial separation and vaginal opening significantly increased in MD (males) and HD (both sexes) and was outside HCD for preputial separation but not vaginal opening F1 pups: viability and weaning indices unaffected In HD mean number of live pups/litter very small but significant decrease on day 14 and 21 with questionable biological significance Pup bw decreased in utero and growth significantly decreased throughout lactation (11-24%) No anogenital distance recorded for F1 pups, preputial separation and vaginal opening significantly increased in correlation with decreased bw No treatment related changes during necropsy in F0 adults or F1 pups F2 pups: viability and weaning indices significantly affected in HD animals, pup mortality greatly increased from PND 0-21, slightly decreased bw but strongly decreased post-natal growth (15-22%) Anogenital distance not affected, no data on preputial separation and vaginal opening because F2 pups were sacrificed on LD21

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						Sign. decreased in-utero growth, post-natal growth and bw during lactation in HD pups Mean age for reaching eye opening in HD pups significantly increased, correlating to decreased bw, no necropsy changes in F1 adults and F2 pups
Anonymous 1997f	(CLH report, 2018; RAC Opinion, 2020)	Rat, preliminary study	0, 18, 35, 70	Gavage	NOAEL: 18	Treatment related decreased parental bw and food consumption at 35 and 70 mg/kg bw/d No offspring anomaly at any dose level
Anonymous 1997f **Nukui and Fujii 1994a**	(CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	mated female Cij:CD(SD) rats (24/group), Prenatal developmental toxicity study (OECD 414, GLP)	0, 5, 16, 50	Gavage (purity 99.46%), GD 6-15	NOAEL for maternal tox: 16 NOAEL for development: 50	No treatment related changes in LD and MD dams Sign. decreased bw gain from GD 6-15 in HD Sign. but small rel. liver and kidney weight increase Total implantations, corpora lutea, live foetuses not affected Resorbed foetuses sign. Increased at 16 mg/kg bw/d without dose correlation No visceral findings, no external test article related malformations but variations like placental and subcutaneous haemorrhage at HD No skeletal malformations, shortened 13th rib (variation) at HD
Anonymous 2008 **Nemec 2003**	(CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	Pregnant female Crl:CD(SD) IGS BR rats (25/dose) 1-gen developmental neurotoxicity study, EPA 870.6300 (1998), GLP	0, 2.5, 10, 45	Gavage, GD 6-21	NOAEL: 2.5 (for maternal toxicity, developmental toxicity and developmental neurotoxicity)	Maternal tox at 45 mg/kg bw/d (single mortality + decreased bw gain and food consumption), not considered as excessive by RAC Decreased pup postnatal survival with total litter loss in three HD dams Pup acoustic startle response with maximum response amplitudes (Vmax) and average response amplitudes (Vave) in HD males significantly reduced compared to the control group values on PND 20 and 60 but with maternal tox, decrease (not significant) also at MD without maternal tox

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						Bw gain of HD males and females sign. Decreased during entire post-weaning period (PND21-72) Time to preputial separation and vaginal opening not affected Deficits (attenuation) in auditory startle response in HD F1 males and females without concomitant effects in other functional endpoints, neuropathology or brain morphometry.
Anonymous 1997e	(CLH report, 2018; RAC Opinion, 2020)	Rabbits, preliminary study, 4 pregnant does/group	0, 5, 13, 30, 75	Gavage, GD6-18		All HD animals dead by day 14 Treatment related reductions in parental bw and food consumption at 13 and 30 mg/kg bw/d No external abnormalities in foetuses at any dose level
Anonymous 1997e **Nukui and Fujii 1994b**	(CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	17 mated female rabbits/group, Prenatal developmental toxicity study (OECD 414, GLP)	0, 7.5, 15, 30	Gavage (purity 99.46%), GD 6-18	NOAEL for maternal tox: 15 NOAEL for development: 30	No increased mortality, toxic signs or organ weight changes in any dose group Number of implantations, corpora lutea, live foetuses unaffected Decreased maternal bw + sign. Decreased food consumption in females at 30 mg/kg bw/d No foetal anomalies on visceral findings despite one HD foetus with microphthalmia, no external findings, no skeletal findings despite some sporadic non-significant differences in the type and incidence of skeletal anomalies in treatment groups without dose dependency

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Kagawa and Nagao 2018	(Anadón et al., 2020)	ICR mice	5	Gavage, GD 6-18		Hypoplasia of the cortical plate and decreased neurogenesis in mice exposed to acetamiprid from embryonic day 6 to 13, whereas newborn acetamiprid-exposed mice showed an abnormal neuronal distribution in the neocortex. Increased numbers of Iba1-immunoreactive and amoeboid-type microglia as well as an increased M1/M2 microglial ratio. Significant neurodevelopmental toxicity and microglial activation in the mouse neocortex
Kagawa and Nagao 2018	(Anadón et al., 2020)	ICR mice	5 (acetamiprid in mixture with imidacloprid)	PD12-PD26		Acetamiprid and imidacloprid impairs neurogenesis and alters microglial profiles in the developing hippocampal dentate gyrus
Nemec 2004	(FAO and WHO, 2012)	Crl:CD(SD)IGS BR rats (12/dose) (GLP)	0, 5, 10, 20, 40	Gavage (purity 100%) GD6 to lactation day 10	NOAEL for maternal tox: 10 NOAEL for development: 20	No increased mortality General physical conditions unaffected Duration of gestation unaffected Mean bw gain and food consumption decreased from 20 mg/kg bw/d Mean numbers of pups born, mean live litter size, percentage of males per litter at birth and postnatal survival were unaffected HD mean pup weights were reduced during PND 4, 7 and 10 General physical condition of F1 pups during postnatal period unaffected Necropsy findings of pups found dead or at scheduled euthanasia on PND21 were normal No observations indicative of neurotoxicity in either the maternal animals or the F1 offspring

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Anonymous 2008 **Nemec 2003**	(CLH report, 2018; FAO and WHO, 2012; RAC Opinion, 2020)	Pregnant female Crl:CD(SD) IGS BR rats (25/dose) 1-gen developmental neurotoxicity study, EPA 870.6300 (1998), GLP	0, 2.5, 10, 45	Gavage (purity >99%) GD 6 to lactation day 21	NOAEL: 10 for maternal tox and developmental tox	One HD female died on GD23 due to dystocia No clinical sings of toxicity Mean bw loss, decreased bw and food consumption in HD, but bw gain increased on lactation days 1-4 No findings at necropsy of F0 females Mean numbers of implantation sites similar to ctrl Mean live litter size 7.3% lower than ctrl in HD and decreased postnatal survival on PND0-1 Total litter loss in three HD females General physical condition of F1 animals normal Offspring bw gains sign. Decreased on PND1 in HD males and females Mean numbers of days to acquisition of balano-preputial separation and vaginal patency not affected For auditory startle response, the maximum response amplitudes (*V*max) and average response amplitudes (*V*avg) were reduced in HD males Locomotor activity, learning and memory in all offspring were unaffected F1 pups necropsy not affected No microscopic findings in the central and peripheral nervous system tissues examined from the selected PND 11 and PND 72 pups Mean absolute and relative brain weights, brain lengths, brain widths and F1 brain morphometry at PND 11 and PND 72 not affected
Sano et al. 2016	(Ongono et al., 2020; X. Zhang et al., 2023)	Male and female C57BL/6J mice (18 in the control group, 19 in the low-dose one and 20 in the high-dose one for the	0, 1, 10	Gavage, GD 6 to lactation day 21, acetamiprid		Male aggressive behaviour – chasing, boxing, wrestling, biting, tail rattling, and offensive lateral attack – significantly increased male aggressive behaviours in the low-dose group only Emotional behaviours using LDT test (light-dark transition test) – for males only, longer time spent in the light compartment in the LDT reflecting not only

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
		assessment of male aggressive behaviour and emotional behaviour; 13 in the control group, 11 in the low-dose one, 13 in the high-dose one for the assessment of behavioural flexibility.				decreased anxiety, but also hyperactivity under specific stressful conditions Spatial learning ability, behavioural flexibility and impulsivity – no differences in spatial learning ability, behavioural flexibility and impulsivity during adulthood in both sex
(Saito et al., 2023a)		Pregnant Female C57BL/6N mice	0.025	Oral (in drinking water), The gestation period (embryonic day 11.5) to maternal ablactation when pups were 4weeks old		Study investigated the effects of acetamiprid (and imidacloprid) on the central nervous system in adult male offspring After maternal ablactation, eight male pups were randomly selected from each group and maintained until 13weeks of age It was performed a behavioural test battery that consisted of the open field (OF) test, the light/dark transition (LD) test, the elevated plusmaze (EP) test, the contextual/cued fear conditioning (FZ) test, and the pre-pulse inhibition (PPI) test No significant differences were observed in the OF and LD test No significant differences were observed in the freezing rate Any changes in the PPI test
(Saito et al., 2023b)		C57BL/6N male mice (with foster females) at PD10 (8/group)	10	Oral (in corn oil), single dose (one group 2 weeks old, second		Study investigated the effects of acetamiprid exposure in early life on brain function in adult mice. The animals were exposed when they were 2 or 11 weeks old. At 12-13 weeks old the mouse behavioural test battery was conducted. The behave. Test battery consisted of the open field test, light/dark transition test, elevated plus-

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
				group 11weeks old)		maze test, contextual/cued fear conditioning test, and pre-pulse inhibition. Open field test – total distance and number of movement episodes did not significantly change in both groups; total center time was significantly higher in 2weeks group, whereas no significant differences observed in the 11weeks group Light/dark transition test – no significant differences among all groups Elevated plus-maze – total distance significantly decreased in the 2 weeks group, whereas no significant differences in 11weeks group Contextual/cued fear conditioning test - significant differences observed in the FZ test in 2weeks group in terms of the freezing rate during conditioning. In the contextual fear test no significant difference in the freezing rate. Pre-pulse inhibition test – none of the groups showed any changes in the PPI test
(Longoni et al., 2024)		Nulliparous Wistar female rats	6	Oral (drinking water) GD2-19		Study tested morphological, biochemical, and neurobehavioral features in preterm foetuses and developing pups after maternal administration of acetamiprid. Results showed no consistent findings indicating maternal, reproductive or developmental toxicity. It was found that acetamiprid has effects on maternal body weight gain, placental butyrylcholinesterase activity, and neurobehavioral responses, suggestive of a mild toxic action.

Table IX.84: Overview of studies with effects on immunotoxicity

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Bown 2010	(FAO and WHO, 2012)	CD-1 mice (10/sex/dose) (GLP)	0, 100, 300 or 900 ppm (0,15.3, 47.0, 128 mg/kg bw/d for males and 0, 20.2, 54.1, 157 mg/kg bw/d for females)	Dietary (purity 99.9%), 4 weeks	NOAEL: >900 ppm (>128/>157 mg/kg bw/d for males and females, respectively) for immunotoxicity	No increased mortality Noisy breathing (rales) in HD males and females Decreased HD bw gain and food consumption Decreased spleen weights in HD females No effect of treatment on the immune system Statistically significant reduction in the observed number of plaque-forming cells per spleen in HD females, correlating with a general reduction in total cell numbers per spleen, showing that the reduced response was due to a reduction in cell number, rather than the immune function of the cells
Moore 2010	(FAO and WHO, 2012)	Sprague-Dawley (Crl:CD (SD) rats (10/sex/dose) (GLP)	0, 100, 300, 900 ppm (0, 7.4, 21.6 and 62.9 mg/kg bw/d for males and 0, 8.6, 24.3 and 67.7 mg/kg bw/d for females)	Dietary (purity 99.9%), 4 weeks	NOAEL: >900 ppm (>62.9/>67.7 mg/kg bw/d for males and females, respectively) for immunotoxicity	No increased mortality Bw gain and food consumption decreased in HD males and females and MD males No effects on spleen and thymus weights or macro pathology No statistically significant changes in the numbers of cells per spleen, plaque-forming cells per 10^6 cells or plaque-forming cells per spleen No effect on immune function, as assessed by the measurement of antigen-specific, T cell–dependent antibody formation
Mondal et al. 2009	(FAO and WHO, 2012)	Female Wistar rats (18/dose)	0, 25, 100, 200	Orally (not further specified) 28 days, 20% formulation without detailed information	Study not used for evaluation	Significant decreases in mean values of total leukocyte count and relative lymphocyte count in HD and of globulin in all treated rats Significant decrease in spleen weight in HD rats Dose dependent depletion of lymphocytes from Malpighian corpuscles in spleens of all treated groups

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Lee et al., 2024		Wistar Rats	40	Dietary, once at G15		Observation of living cultured microglia and their reactivity to inflammation via the acoustic impedance mode of a scanning acoustic microscope Primary cultured microglia collected from rat pups exposed in utero Acetamiprid induced microglia response

Table IX.85: Overview of studies on neurological and developmental effects in vitro

Reference	Cited in	Findings
Cartereau et al. 2020	(NTP, 2020)	Cell types - Frog oocytes expressing rat recombinant nAChR Concentrations tested - 0.00001 to 0.01 M Effects on ACh-induced currents
Camlica et al. 2018	(NTP, 2020)	Cell types - Frog sciatic nerves Concentrations tested - 1, 10, 100, 1000 µM Effects on nerve action potential amplitude and area; nerve histopathology
Christen et al. 2017	(NTP, 2020)	Cell types - Rat PC12 cell line Concentrations tested - 1, 10, 100 µM Effects on neurite outgrowth; gene expression
Gyori et al. 2017	(NTP, 2020)	Cell types - Purified electric eel AChE Concentrations tested - 1 µM to 1 mM (specific concentrations not reported for each compound) Effects on Inhibition of AChE activity; AChE binding kinetics
Kimura-Kuroda et al. 2012	(NTP, 2020)	Cell types - Rat neonatal cerebellar neurons Concentrations tested - 1, 10, 100 µM Effects on Ca2+ influxes; neural excitation
Kimura-Kuroda et al. 2016	(NTP, 2020; Phogat et al., 2022)	Cell types - Rat neonatal cerebellar neurons Concentrations tested – 1 µM, 14 days Effects on gene expression; neuron branching
Senyildiz et al. 2018	(NTP, 2020; Phogat et al., 2022)	Cell types - Human neuroblastoma cell line (SH-SY5Y) Concentrations tested – 0.125, 0.25, 0.5, 1, 2, 4 mM (cytotoxicity, cell viability) 50, 100, 200, 500 µM (DNA damage) Effects on Cytotoxicity; DNA damage
Babelova et al. 2017	Zhao et al. 2020, (NTP, 2020; X. Zhang et al., 2023)	Cell types - Fertilized mouse and rabbit embryos Concentrations tested – 0.1, 1, 10, and 100 µM Effects on Embryo development; blastomere number; cell death; blastocyst quality
Gu et al. 2013	(NTP, 2020; RAC Opinion, 2020)	Cell types - Mouse oocytes, sperm, fertilized zygotes, fertilized embryos, female B6D2F1 mice as oocyte donors, male B6D2F1 mice as semen donors Concentrations tested – 0.5, 5 mM All fertilized oocytes survived without evident changes in cell morphology Motility of spermatozoa showed no obvious difference from that of controls Percentage of DNA fragmented spermatozoa was similar across all treatment groups Effects on sperm DNA integrity; sperm fertilization capability; embryo development; zygote development
(Guzman-Vallejos et al., 2024)		Neuroblastoma SH-SY5Y cells Concentration 10 µM and 500 µM Docking analysis showed that the binding of ACE occurred at the same aromatic cage that the specific α-7 nAChR agonist EVP-6124

Table IX.86: Overview of in vitro studies on ROS generation

Reference	Cited in	Findings
Annabi et al. 2019	(Phogat et al., 2022; Zuščíková et al., 2023)	Cell types - Pheochromocytoma adrenal medulla cells (PC12) Concentrations tested - 100–700 µM Effects on ↑ malondialdehyde levels and ROS generation; loss of mitochondrial membrane potential
Kara et al. 2020	(Phogat et al., 2022; Zuščíková et al., 2023)	Cell types - Pancreatic cell line (AR42J) Concentrations tested - 1–50 mM (acetamiprid) Effects on reduction in glutathione levels
Gomez et al. 2020	(Phogat et al., 2022; Zuščíková et al., 2023)	Cell types - Isolated trophoblast cells (HTR-8/SVneo) Concentrations tested – 0.1 - 100 µM for 4 and 24 hours Effects on ↑ ROS production and superoxides; ↓Glutathione S-transferase, catalase, and superoxide dismutase Cytotoxic for human trophoblasts, and oxidative stress is a toxicity mechanism
Babel'ová et al. 2017	(Zuščíková et al., 2023)	Negatively affected embryonic development Decreased quality of blastocysts
Çavaş et al. 2012	(Phogat et al., 2022)	Human colon carcinoma cells (CaCo-2) 25–350 µM, 24 h (acetamiprid) Decreased cell viability and NDI; increased binucleated cell count, GDI, MN formation and single and double strand DNA break in concentration dependent-manner
Çavaş et al. 2014	(Phogat et al., 2022)	Human lung fibroblast cells (IMR-90) Acetamiprid, 50–1600 µM, 24 h Increased frequency of MN formation and GDI; induced single and double strand DNA breakage; decreased cell survival at all concentration
Kocaman and Topaktaş 2007	(Phogat et al., 2022)	Human blood lymphocytes 25, 30, 35 and 40 µg/mL, 24 and 48 h Induced CA, abnormal SCE, MN formation and declined MI, PI and NDI expression (significant change observed at 48 h exposure only)
Kocaman and Topaktaş 2010	(Phogat et al., 2022)	Human blood lymphocytes Mixture of ACMP and Cypermethrin (12.5+2.5, 15+5, 17.5+7.5 and 20+10 µg/mL), 24 and 48 h Increased frequency of SCE, MN formation and CA; decreased PI, MI, NDI and induced lymphocyte's genotoxicity
Muranli et al. 2015	(Phogat et al., 2022)	Human blood lymphocytes Individual and combined treatment of ACMP (0.625, 1.25 and 2.5 µg/mL)+Propineb (12.5, 25 and 50 µg/mL), 24 and 48 h No cytotoxic effects in single (24 and 48 h) or combined (24 h) exposure except 1.25 µg/mL acetamiprid exposure Acetamiprid and propineb didn't increase significant MN formation except for 12.5 µg/mL propineb treatment for 24 h. However, the combined treatment significantly increased MN frequency at 48 h exposure.
Arican et al. 2019	(Phogat et al., 2022)	Male Sprague Dawley rats Acetamiprid, 12.5, 25 and 35 mg/kg b.wt, orally, 90 days Reduced 5- methyl cytosine levels in liver in dose dependent manner and in brain tissue only at high dose.

Reference	Cited in	Findings
		Decreased gene expressions of DNMT1, DNMT3a and DNMT3b (DNA methyltransferase) in liver and brain tissues in order of DNMT3a>DNMT1>DNMT3b
Bagri and Jain 2019	(Phogat et al., 2022)	Mice 2.3 and 4.6 mg/kg b.wt, i.p., 60 days, acetamiprid Caused chromosomal breakage, fragmentation and chromatids gap; significantly increased MN frequency in polychromatic and normochromatic erythrocytes of bone marrow
Wang et al. 2023		HepG2 cells exposed to acetamiprid 500mM The expression of biomarkers related to oxidative stress, apoptosis and cell necrosis was examined EC50 112.61 μM Disrupted redox homeostasis
Loser et al., 2021		SH-SY5Y neuroblastoma cells, LUHMES neuronal precursor cells Triggered [Ca2+]i signalling at 10–100 μM Strong synergy of the active neonicotinoids (at low micromolar concentrations) with the α7 nAChR-positive allosteric modulator PNU-120596 was observed in LUHMES and SH-SY5Y cells, and specific antagonists fully inhibited such signalling.
Öztaş et al., 2021		The effects of acetamiprid on oxidative stress, endoplasmic reticulum (ER) stress, cellular death, mRNA expression levels of related genes and protein expressions of related molecular mechanisms in SH-SY5Y human neuroblastoma cells IC50 value of acetamiprid was determined as 4.26 mM after 24 h of treatment by MTT assay Increase in reactive oxygen species (ROS) production and calcium release Significant increases were measured in inositol-requiring enzyme 1-alpha (IRE1-α) and binding immunoglobulin protein 90 (GRP90) levels as well as mRNA expression levels of caspase 3, 4 and 9 genes indicating enhanced ER stress. Apoptosis and ER stress-related genes were significantly upregulated at ≥2 mM
Ramirez-Cando et al., 2023		Human neuroblastoma SH-SY5Y cells Acetamiprid concentration 0.01–0.5 mM Did not have acute cytotoxic effects in both non-differentiated and in staurosporine-differentiated SH-SY5Y cells cytotoxicity as measured by the MTT and vital-dye exclusion tests. During differentiation dose-dependently caused neurite branch retraction on day 3, likely because of oxidative stress, to the extent that cells turned into spheres without neurites after 7-day treatment
X. Wang et al., 2022		Neuro-2a cells; concentrations 76.3 μM (ACE10) and 267.1 μM (ACE20) Distinct elevation of lactate dehydrogenase (LDH) activity and caspase 7 level demonstrated the influences on necrosis and apoptosis Remarkable decrease of lipid hydroperoxides (LOOH) and increase of reactive oxygen species (ROS) generation were found only in the ACE20 group. Interference with glutathione metabolism pathway was further validated by detecting GPx (glutathione peroxidase), GSH (reduced glutathione) and GSSG (oxidized glutathione) levels

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Part X

Substance monograph for **imidacloprid** and derivation of HBM guidance values (HBM-GVs) for the general population

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Responsible author(s)	Falko Partosch (Ramboll) on behalf of UBA
Co-authors	Petra Apel/ UBA/ petra.apel@uba.de
Internal Reviewers[2]	WP4 co-leaders
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Abstract

Imidacloprid is a neonicotinoid insecticide. It was approved as active ingredient of plant protection products in the European Union in 2013 but was restricted by the European Commission in 2018 due to its adverse effects on honeybees.

Imidacloprid is also included in Annex I to Directive 98/8/EC of the European Parliament and of the Council as an active substance for use in biocidal products of product-type 18. This approval should actually expire on 30 June 2023 but the expiry date of the approval was postponed to 31 December 2025 (COMMISSION IMPLEMENTING DECISION (EU) 2023/460 of 2 March 2023).

Imidacloprid has raised concerns regarding its potential adverse effects on human health. For this reason, it (and its metabolites) was included in the recommended list of exposure biomarkers in PARC-aligned studies in children and adults. Consequently, Human Biomonitoring Guidance Values (HBM-GVs) were derived to be able to carry out a health-based interpretation of the human biomonitoring (HBM) results.

A systematic literature search was conducted using PubMed and Scopus. Until 3rd April2024, 2534 publications were found, of which 227 were considered relevant - provided results specifically for imidacloprid on biomonitoring and epidemiological studies as well as toxicology. The most relevant ones (n=191) were further evaluated. In addition, existing European and international authority reports were gathered and the reported toxicological reference values were taken into consideration. The most recent literature highlights a number of additional studies; however, many of these exhibit methodological limitations, focus on highly specific toxicological endpoints, or fail to demonstrate lower NOAELs. Consequently, the Acceptable Daily Intake (ADI) value of 0.06 mg/kg bw/d, established by EFSA, was used for the derivation of HBM-GV_{GenPop}.

The most critical effects were observed in chronic carcinogenicity studies conducted in animals (rats), and concerned specifically non-neoplastic lesions (decreased body weight gain, changes in clinical chemistry parameters related to hepatotoxicity, and a dose dependent thyroid mineralization). Related to these adverse effects the ADI of 0.06 mg/kg bw/d was derived by EFSA served as the toxicological (toxicity) reference value (TRV). The literature search and analysis carried out did not produce any results that would call this ADI into question.

The urinary mass balance approach with the specific metabolite imidacloprid olefin (IMI-olefin) was used to derive HBM guidance values (HBM-GVs) for the general population. This resulted for IMI-olefin in HBM-GVs for children and men of 0.54 mg/L urine and for women of 0.46 mg/L urine.

Key Words

HBM guidance values, HBM-GV, general population, neonicotinoids, imidacloprid, 4-/5-OH-imidacloprid, imidacloprid-olefin, insecticides

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Authors and Acknowledgements

Authors

Falko Partosch on behalf of UBA

Petra Apel, UBA, Germany

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Abbreviations and Acronyms

6-CNA	6-Chloronicotinic acid
6-CNA-gly	6-Chloronicotinic acid glycine conjugate
AAOEL	Acute Acceptable Operator Exposure Level
AChE	Acetylcholinesterase
AChR	Acetylcholine Receptors
ACP	Acid Phosphatase
ADI	Acceptable Daily Intake
AdjOR	Adjusted Odds Ratio
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AOEL	Acceptable Operator Exposure Level
AOPP	Advanced Oxidation Protein Products
ARfD	Acute Reference Dose
ASG	Atypical Symptomatic Group
AST	Aspartate aminotransferase
AUC	Area Under the Curve
BASC	Behavior Assessment System for Children
BChE	Butyrylcholinesterase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
BW	Body Weight
CA	Chromosomal Aberration
CAS	Chemical Abstracts Service
CAT	Catalase
CHAMACOS	Center for the Health Assessment for Mothers and Children of Salinas
CHD	Congenital Heart Diseases
CHO	Chinese Hamster Ovary
CI	Confidence Interval
CKD	Chronic Kidney Disease
CLO	Clothianidin
c_{max}	Maximum Measured Concentration
DF	Detection Frequencies
DHC	Dehydrocorticosterone
DHEA	Dehydroepiandrosterone
DIN	Dinotefuran
dme-ACE	N-desmethyl-acetamiprid

DNA	Deoxyribonucleic Acid
DN-IMI	Desnitro-imidacloprid
DTH	Delayed Type Hypersensitivity
EC	European Commission
ECHA	European Chemicals Agency
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EPA	Environment Protection Agency
ESB	German Environmental Specimen Bank
F0, F1	Filial generations
FAI	Free Androgen Index
FAO	Food and Agriculture Organization
FLUP	Flupyradifurone
FOB	Functional Observational Battery
FSH	Follicle-Stimulating Hormone
GD	Gestation Day
GI	Gastrointestinal
GLP	Good Laboratory Practice
GOT	Glutamic Oxaloacetic Transaminase
GPT	Glutamic Pyruvic Transaminase
GPx	Glutathione Peroxidase
GSH	Glutathione
GTCS	Generalized Tonic Clonic Seizure
HBM	Human Biomonitoring
HBM-GV	Human Biomonitoring Guidance Value
HCD	Historical Control Data
HD	High Dose
HFD	High Fat Diet
HPA	Hypothalamic Pituitary Adrenal
IARC	International Agency for Research on Cancer
IMI	Imidacloprid
IMIeq	Imidacloprid Equivalent Total Neonicotinoids
IQR	Interquartile Range
IRR	Incidence Rate Ratio
IUPAC	International Union of Pure and Applied Chemistry
LC50	Lethal Concentration 50%
LD	Low Dose

LD50	Lethal Dose 50%
LH	Luteinizing Hormone
LMI	Lean Mass Index
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
LOQ	Limit of Quantification
LPO	Lipid Peroxidation
MD	Mid Dose
MDA	Malondialdehyde
MOA	Mode of Action
NAC	N-Acetyl-L-cysteine
NEOs	Neonicotinoids
NHANES	National Health and Nutrition Examination Survey
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NSG	Non-Symptomatic Group
OECD	Organisation for Economic Co-operation and Development
OP	Osteoporosis
OPPTS	Office of Prevention, Pesticides and Toxic Substances
OR	Odds Ratio
P0, P1	Parental generations
PCNA	Proliferating-Cell Nuclear Antigen
PI	Ponderal Index
PND	Postnatal Day
POD	Point Of Departure
PPAR	Peroxisome Proliferator-Activated Receptor
PU	Polyurethane
PXR	Pregnane X receptor
QSAR	Quantitative Structure Activity Relationship
RAC	Risk Assessment Committee
RBC	Red Blood Cell
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
ROS	Reactive Oxygen Species
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvate Transaminase
SOD	Superoxide Dismutase
SULF	Sulfoxaflor

TBIL	Total Bilirubin
TC	Total Cholesterol
TDI	Tolerable Daily Intake
TG	Test Guideline
THIAC	Thiacloprid
THIAM	Thiamethoxam
TL	Telomere Length
tmax	Time to maximum concentration
TML	Thymol
tRNA	Transfer Ribonucleic Acid
TRV	Toxicity Reference Value
TSG	Typical Symptomatic Group
TTEF	Transplacental Transfer Efficiencies
Vmax	Maximum Response Amplitudes
WHO	World Health Organization
WtHR	Waist to Height Ratio

1 Introduction

Imidacloprid (IMI) is a neonicotinoid insecticide that was launched on the global market in 1991 (Zhang and Lu, 2022). It is designed to be effective by contact or ingestion and acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013). Nicotinic acetylcholine receptors (nAChRs), which are imidacloprid targets, are present in both insect and mammalian nervous systems. However, the specific action of imidacloprid on insect nAChRs makes it effective as an insecticide.

The substance is used to control sucking insects, some chewing insects including termites, soil insects, and fleas on pets. In the European Union, imidacloprid's use has been severely restricted due to environmental concerns, particularly its harmful effects on pollinators (in 2013 restrictions on the use of plant protection products and treated seeds containing three specific neonicotinoids: clothianidin, imidacloprid, and thiamethoxam, Regulation (EU) No 485/2013). Since 2018, EU regulations have banned all outdoor uses of imidacloprid in agriculture, restricting its application exclusively to permanently enclosed greenhouses where the exposure of pollinators and other wildlife is minimized (Regulation (EU) 2018/783)[57].

For these restricted uses, imidacloprid, like other neonicotinoid insecticides , is applied by foliar spraying to above-ground plants, root drench to soil or trunk injections only in specific and controlled environments, like closed greenhouses, to prevent exposure to non-target species (CLH report, 2018).

Imidacloprid is also included in Annex I to Directive 98/8/EC of the European Parliament and of the Council as an active substance for use in biocidal products of product-type 18. This approval should actually expire on 30 June 2023 but the expiry date of the approval was postponed to 31 December 2025 (COMMISSION IMPLEMENTING DECISION (EU) 2023/460 of 2 March 2023).

The active substance imidacloprid has raised concerns regarding its potential adverse effects on human health. For this reason, it (and its metabolites) was included in the recommended list of exposure biomarkers in PARC-aligned studies in children and adults. Consequently, Human Biomonitoring Guidance Values (HBM-GVs) were derived for the general publication to be able to carry out a health-based interpretation of the human biomonitoring (HBM) results.

[57] http://npic.orst.edu/factsheets/archive/imidacloprid.html#references

2 Methodology

Relevant agency reports and risk assessments for imidacloprid were identified through general desk research. This research focused specifically on human health effects and exposure levels. The process involved gathering information from official websites of organizations and institutions. Reports from EFSA[58], ECHA, WHO, and NTP were included.

To further substantiate the findings from the agency reports, a systematic literature review was conducted to identify all relevant data for the derivation of HBM-GVs. Although no official guideline for systematic literature search was followed, the work was conducted in line with standard procedures for systematic literature search. The main goals of the literature review were to identify:

- What are the most relevant adverse effects of imidacloprid in humans?
- What is the lowest threshold value to be used as the point of departure (POD) for the derivation of HBM-GVs?
- What is the metabolic pathway of IMI in the human body and its main biomarkers?
- What is the excretion range of IMI and its most relevant biomarkers?

For a systematic literature search of scientific publications, a search string was developed consisting of (A) a health part and (B) synonyms for imidacloprid and its major metabolites (Table X.1). The full search term is shown in the Appendix.

Table X.87: Parts of the search term used to search for scientific publications

(A) Health part	health OR adverse OR toxic* OR neurotox* OR epidem* OR cohort OR genotox* OR genetic* OR carc* OR cancer OR reproduc* OR reprotox* OR developmental OR fertil* OR disease OR neurologic* OR metabol* OR adme OR pbpk OR pbtk OR excret* OR {in vivo} OR {in vitro} OR {in silico} OR endocrine OR {half-life} OR immun* OR moa OR {mode of action}
(B) Synonyms for Imidacloprid and its major metabolites	Imidacloprid OR "IMI" OR "6-Chloronicotinic acid" OR "6-CNA OR "4-imidazolidine" OR "5-imidazolidine" OR "olefinic imidacloprid" OR "desnitro imidacloprid" OR "hydroxy imidacloprid" [OR *additional synonyms of all substances*]

The search was carried out in SCOPUS and Pubmed using the same search string in both databases and revealed a total number of 2534 publications that were published from1st January 2020 until 3rd April 2024. The publications were screened for relevance according to inclusion and exclusion criteria described below and based on title and abstract using DistillerSR. In this first step, 2307 publications were excluded. The remaining 227 publications were screened by full text and relevant information is discussed further in the following chapters of this report. Overall, 191 publications were included.

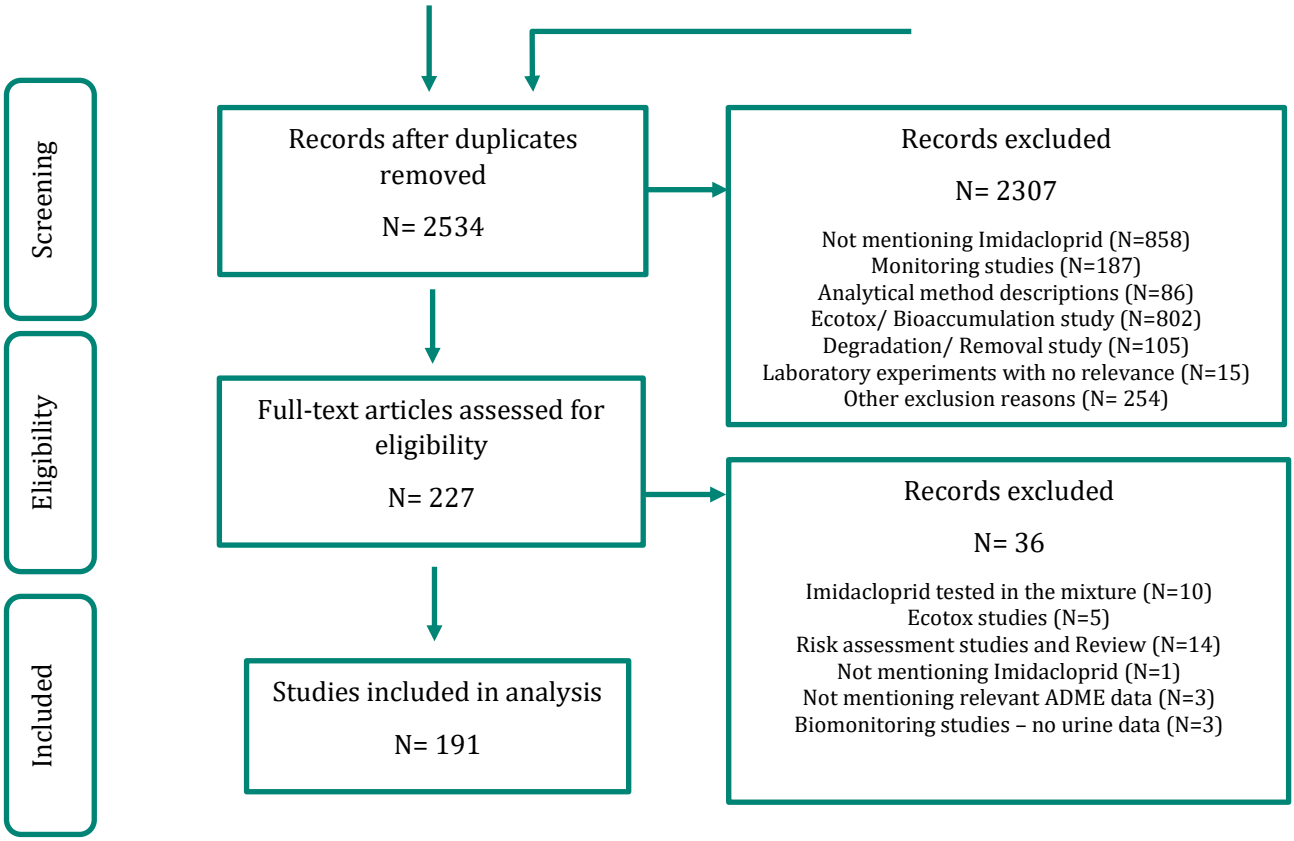
The inclusion and exclusion criteria used by the subject matter experts are described in Table X.88.

[58] Sometimes EFSA has cited WHO, so that WHO is also given here as the literature source and not the original publication.

Table X.88: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Studies on mammals (the relevant toxicological studies in vivo)	Studies on non-target vertebrate species (e.g. fish, birds)
Human biomonitoring studies	Monitoring studies
Epidemiological studies	Chemical distribution in natural media (water, soil, air) and residue not measured in the organism
Studies with relevant information for the deviation of human biomonitoring guidance values such as excretion concentration fraction, toxicokinetic data	Reports of animal deaths by poison, which lacks a usable concentration and/or duration
	Substance tested in mixture only; no single chemical reported
	Field studies with no clear link to the exposure, side effect
	Measured chemical present in organism, but lacking quantification of exposure; lacks usable concentration and/or duration; these are only in the field
	Analytical methods, removal, laboratory experiments
	Toxicological studies in vitro, in silico

The following PRISMA diagram shows the process graphically.

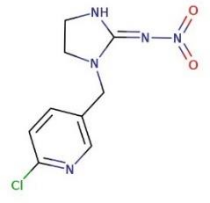


3 General characteristics

3.1 Physico-chemical properties

The information on substance characteristics and physico-chemical properties of imidacloprid is based on the corresponding REACH registration dossier[59].

Table X.89: Substance characteristics and physico-chemical properties of imidacloprid

Name	Imidacloprid
IUPAC names	1-(6-chloropyridin-3-ylmethyl)-N-nitroimidazolidin-2-ylidenamine
EC no.	428-040-8
CAS no.	138261-41-3
Sum formula	$C_9H_{10}ClN_5O_2$
Structural formula	
Appearance [1013 hPa; at 20°C]	solid: particulate/powder
Molecular weight [g/mol]	255.7
Relative density [g/cm³; at 20°C]	1.52
Melting point [°C; at 1013.3 hPa]	142.6
Boiling point [°C; at 1013.3 hPa]	> 220
Vapour pressure, hPa [at 20°C]	0
Log Pow [at 24°C]	0.7
Water solubility [mg/L; at 20°C]	610
Dissociation constant [pK_a (= -log K_{10a}) at 23°C]	11.8

[59] Imidacloprid ECHA CHEM: https://chem.echa.europa.eu/100.102.643/overview?searchText=138261-41-3 (accessed on 06.03.2025)

3.2 Use of the substance

Imidacloprid is an active ingredient regulated as both as plant protection product and as biocide (insecticide PT18) in the European Economic Area (EEA). Imidacloprid-containing biocidal products are registered for professional use, including by farmers and pest control operators, and are formulated as baits to control insect pests such as house flies and cockroaches (CLH report, 2018).

In 2013, imidacloprid, among others, was approved as active substance in the EU under the Plant Protection Products Regulation (EC) No 1107/2009 but its use was severely restricted and these restrictions were tightened even further by the European Commission in 2018 to protect honeybees based on a risk assessment of the EFSA in 2013. Imidacloprid, clothianidin and thiamethoxam are banned, except in closed permanent greenhouses. As a result of these restrictions, the applicants (manufacturers) withdrew their application for renewal of approval and the registration of imidacloprid in the EU expired on December 1, 2020[60].

The substance is registered under REACH Regulation and is manufactured in or imported into the European Economic Area in quantities of ≥ 1 000 tonnes per year. It is used in formulations or repacking and in the manufacture of plant protection products or biocide products[61].

Imidacloprid is widely used in veterinary medicine for the topical treatment of fleas in animals, providing effective control e.g. for dogs, cats. Veterinary products containing imidacloprid, such as 'Advantage' or 'Imoxat', are applied directly to the skin[62].

Imidacloprid has a harmonized classification to annex VI of the CLP regulation with Acute Tox. 3; H301 (Toxic if swallowed), Aquatic Acute 1; H400 (Very toxic to aquatic life) and Aquatic Chronic 1; H410 (Very toxic to aquatic life with long lasting effects)[63].

3.3 Possible route of exposure

The general population is mainly exposed through food and drinking water (Zhang and Lu, 2022). Ingestion is thus the primary route of exposure. In the latest EFSA report on residues in food, it was shown that imidacloprid appeared in 3 samples, although the maximum residue levels were not exceeded[64].

For the occupational population, including farmers, their families if they apply pesticides or biocides, the workers, etc., the primary route of exposure is skin (approx. 90%) and inhalation (approx.10%) (Cao et al., 2015; US EPA, 2017).

[60] Neonicotinoids - European Commission (europa.eu) (accessed on 10.07.2024)
[61] https://echa.europa.eu/de/substance-information/-/substanceinfo/100.102.643 (accessed on 10.07.2024)
[62] EMA https://www.ema.europa.eu/en/search?f%5B0%5D=ema_search_entity_is_document%3ADocument&search_api_fulltext=imidacloprid (accessed on 10.03.2025)
[63] https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/22870 (accessed on 10.07.2024)
[64] EFSA https://multimedia.efsa.europa.eu/pesticides-report-2021/ (accessed on 10.03.2025)

4 Existing toxicological reference values (TRV)

The following table X.4 summarises available toxicological reference values (TRV) for imidacloprid proposed by EFSA (2013).

EFSA's ADI of 0.06 mg/kg bw/d has been chosen as basis for the derivation of HBM-GVs (see chapter 6). The reasons are as follows:

- It is based on a chronic toxicity/carcinogenicity study conducted following the OECD test guideline 453 (the other values from table X.4 are sub-acute and thus not suitable to derive a lifelong or chronic guidance value).
- No other test results from epidemiological studies or animal studies published after 2013 have been identified that would call the chosen NOAEL and thus the ADI into question.

Table X.90: Available toxicological (toxicity) reference values (TRVs)

Agency	Type of value	Value	Key study	Exposure route	Critical effect	POD	AF	Source
EFSA 2013	ADI	0.06 mg/kg bw/d	Rat 2-year study (Eiben, 1991 and Eiben and Kaliner, 1991 as cited in FAO and WHO, 2002).	Oral	Reduced body weight gain, liver (blood chemistry), thyroid (mineralisation in the follicular colloid)	NOAEL = 5.7 mg/kg bw/d	100	(EFSA Panel on Plant Protection Products and their Residues (PPR), 2013)
	AOEL	0.08 mg/kg bw/d	Dog, 28 days (Bloch et al. 1987 as cited in FAO and WHO, 2002) and 90 days (Ruf 1990 as cited in FAO and WHO, 2002)	Oral	Hepatocellular hypertrophy and follicular atrophy of the thyroid, CNS effects (ataxia, tremors)	NOAEL = 7.8 mg/kg bw/d	100	(EFSA Panel on Plant Protection Products and their Residues (PPR), 2013)[a]
	ARfD	0.08 mg/kg	Dog, 28 days (Bloch et al. 1987 as cited in FAO and WHO, 2002) and 90 days (Ruf 1990 as cited in FAO and WHO, 2002)	Oral	Hepatocellular hypertrophy and follicular atrophy of the thyroid, CNS effects (ataxia, tremors)	NOAEL = 7.8 mg/kg bw/d	100	(EFSA Panel on Plant Protection Products and their Residues (PPR), 2013)

[a] In the 2013 report it is mentioned: As the current ARfD and AOEL for imidacloprid may not be protective enough for potential developmental neurotoxicity of this active substance, the Panel also recommends to conservatively lower these reference values to the same level as the ADI (0.06 mg/kg bw)

5 Toxicological profile

This toxicological profile aims to synthetize findings from various studies, including data from major agencies such as NTP, EFSA, and other relevant literature. The last comprehensive review by the NTP was completed in 2020. Therefore, a literature review was conducted to update the profile with studies published since 2020. The newly identified studies (since 2020) have been categorized into the following subchapters related to findings in humans (toxicokinetic, metabolism, biomonitoring, epidemiological studies) as well as findings in animals (toxicokinetic, neurotoxicity, repeated dose studies and effects on fertility). The following chapters are discussing the findings in more detail.

5.1 Findings in humans

Information on single and repeated exposure, genetic toxicity, carcinogenicity, or reproductive toxicity in humans is limited. Cases of poisoning are described below in chapter 5.1.2.

5.1.1 Toxicokinetics and metabolism

Numerous studies describe that imidacloprid (IMI) is rapidly hydroxylated to 4- or 5-OH-imidacloprid and further metabolized to IMI-olefin (for more information see Table VI.35 in the Appendix). According to (Wrobel et al., 2023), the recommended biomarker for assessing the exposure to IMI is IMI-olefin in urine. While hydroxylated metabolites 4-/5-OH-IMI in urine were also considered good and robust biomarkers of exposure, the analytical method used by (Wrobel et al., 2023) does not separate the two isomers, and the detector response for these analytes might differ to some extent. Therefore, using the reported Fue for the hydroxylated metabolites for reverse dosimetry requires the specific use of the "Wrobel, 2023 method".

There are results of two quantitative toxicokinetic studies in humans available which have been reviewed and approved by the relevant ethics committees in accordance with internationally recognised standards (Harada et al., 2016; Wrobel et al., 2023). The other studies identified may provide supplementary information, such as biomarker levels in urine, excretion concentration ranges of biomarkers; however, the two studies selected are the most suitable for the assessment, as they offer the most comprehensive data required for the calculation of the human biomonitoring guidance value (HBM-GV) such as urinary excretion fraction (Fue). In the first human toxicokinetic study, a micro dose of a mixture of deuterium-labelled neonicotinoids (5 µg each of acetamiprid-d6, clothianidin-d3, dinotefuran-d3, and imidacloprid-d4) was orally ingested by each of the nine healthy adults. For a period of 4 days, 24 h pooled urine samples were collected from each individual. Most of the ingested imidacloprid was metabolised into unidentified metabolites, the amounts being converted to the two major specific metabolites are not given in the publication. The authors of the study state that exposure to metabolites and the toxicities of the metabolites to humans were not within the scope of that study. Only 12.7 % of the imidacloprid dose was excreted unchanged in urine within 96 hours (Harada et al., 2016).

In the study of (Wrobel et al., 2023) the metabolism and the urinary excretion of imidacloprid was investigated after a single oral dose at the current ADI as set by EFSA, which corresponded to a total dose of 5 mg in three male volunteers for imidacloprid. Post-dose urine samples were collected consecutively and completely up to 48 h. During the analysis, the parent compound and three putative metabolites, hydroxylated IMI (4-/5-OH-IMI), IMI-olefin and 6-CNA-glycine were detected in the post-dose urine samples. However, the third metabolite 6-CNA-glycine is not specific to imidacloprid and, with a calculated Fue of 0.48 ± 0.05% after 48 hours, must be also considered a minor metabolite of IMI compared to 4-/5-OH-IMI (Fue = 31 ± 4%) and IMI olefin (Fue = 27 ± 8%). The Fue values for IMI-olefin in urine and the hydroxylated metabolites 4-/5-OH-IMI in urine varied inter-individually by a factor of 2.2 and 1.4, respectively, so that the Fue values obtained can in principle be used for reverse dosimetry. A prolonged excretion of unchanged IMI up to 48 h was observed which is in line with the results reported by Harada et al. (2016). The urinary excretion fraction for IMI after 48 h was 8.3 ± 0.6%. For IMI, 4-/5-OH-IMI and IMI-olefin the Cmax values were 560 ± 47, 727 ± 141 and 557 ±197 µg/g creatinine, respectively. Tmax for IMI, 4-/5-OH-IMI and IMI-olefin was 2.0 ± 0.4, 7.9 ± 2.7 and 8.4 ± 2.5 h, respectively; Values for the elimination half-life were 12.2 ± 2.0, 22.7 ± 9.2 and 12.4 ± 1.5 h, respectively (Wrobel et al., 2023).

Because the study of (Wrobel et al., 2023) is the only one that presented urinary excretion fractions for IMI and its major metabolites (4-/5-OH-IMI, IMI-olefin), the data will be used for the further derivation of the HBM-GVs. However, since the analytical method used by (Wrobel et al., 2023) does not separate the two isomers 4-/5-OH-IMI, and the detector response for these analytes might differ to some extent, only the 48 h excretion fractions for IMI-olefin in the amount of 27 % are used in the derivation of HBM-GVs (see chapter 6.2).

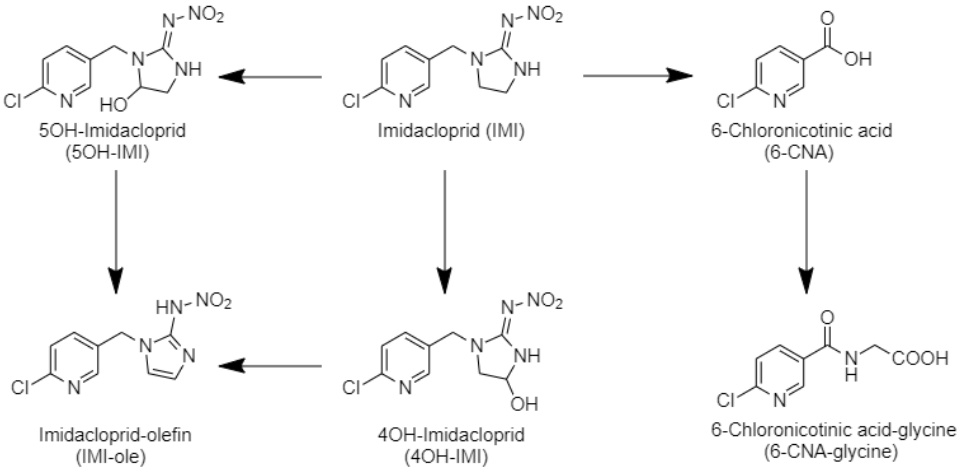


Figure X.10: Proposed metabolic transformation pathway for imidacloprid (M=255.66 g/mol): 4-/5-OH-imidacloprid (271.66 g/mol); imidacloprid-olefin (M=253.66 g/mol); 6-Chloronicotinic acid (M=157.55 g/mol); 6-Chloronicotinic acid-glycine (M=214.55 g/mol) 4-/5-OH-IMI and IMI-ole are specific metabolites of the parent compound (Wrobel et al., 2023).

5.1.2 Health Effects

Several epidemiological studies are available after exposure to imidacloprid. Within the literature search 40 epidemiological studies were found, 25 of which were published since 2020. These studies include 5 case control studies, 22 cross-sectional studies, 1 prospective cohort study, 11 case reports (poisoning), and 1 review. Most of the studies were carried out in the USA and the Asia. However, in most cases, there is not only exposure to imidacloprid alone, but exposure to several neonicotinoids. Due to these co-exposures and based on the fact that the studies focused on different health endpoints, there is no clear determination of the most predominant and common effects (such as neurodevelopmental outcomes: autism spectrum disorder and intellectual disability; reproductive and birth outcomes: congenital heart defects, birth defects, birth size parameters; metabolic and endocrine outcomes: gestational diabetes mellitus, testosterone levels, insulin and glucose parameters and obesity; cancer outcomes – liver cancer; and others such as osteoporosis, renal dysfunction, serum lipid profiles) related to exposure to imidacloprid.

In some case studies, accidental or suicidal acute exposure to high doses of several neonicotinoids and imidacloprid alone with unspecific symptoms such as headache, general fatigue, palpitations, chest pain, abdominal pain, muscle pain/weakness/spasms, and cough were reported. In addition, acute poisoning symptoms such as nausea, vomiting, consciousness disturbance, hypotension, hyperglycaemia, tachycardia, hypoxia, and convulsions were observed.

Aside from case reports, the largest pocket of human epidemiological evidence consisted of four studies reporting developmental or congenital outcomes after long-term exposure (Carmichael et al. 2014; Shaw et al. 2014; Yang et al. 2014 and Marfo et al. 2015 cited in (NTP, 2020)). Carmichael et al. (2014) conducted a case-control study in California involving infants or fetuses with congenital heart defects, utilizing state pesticide use records and proximity of residence from 1997-2006. Although the study estimated exposure categorically and derived adjusted odds ratios (AdjORs) for imidacloprid and neonicotinoid pesticides, direct exposure assessments were lacking, leading to insufficient evidence for conclusions. Shaw et al. (2014) and Yang et al. (2014) both conducted California-based case-control studies on birth defects (gastroschisis and anencephaly/spina bifida/cleft palate), estimating exposure from pesticide records. Both studies faced limitations of direct exposure assessments, relying on model-based estimations without solid evidence to link neonicotinoid exposure and birth defects. Marfo et al. (2015)

monitored multiple insecticides, including imidacloprid, in urine samples of patients near agricultural fields in Japan. Neonicotinic symptoms arose in subjects with high levels of thiame-thoxam and acetamiprid metabolites, although timing of exposure was unknown, and imidacloprid was not detected in samples, rendering the conclusions ambiguous.

Godbole et al. (2022) studied 5-hydroxy-imidacloprid urinary concentrations and associated adiposity measures among US adults. Despite finding associations with increased rates of overweight/obesity, limitations included cross-sectional study design, potential misclassification bias, short neonicotinoid half-lives, and self-reported data leading to inconclusive temporal associations.

Case-control studies focused on mothers during pregnancy to estimate exposures to imidacloprid and neonicotinoids and reported on different types of birth defect outcomes or the occurrence of autism spectrum disorder (ASD) in participating children (Shaw et al. 2014; Yang et al. 2014; Carmichael et al. 2014; Von Ehrenstein et al. 2019; Keil et al. 2014). The studies investigating the exposure to neonicotinoids (NEOs) and metabolites with children's adiposity did identify contradictory results (positive, negative, no association). Only the cross-sectional study measured imidacloprid urinary concentrations and evaluated biochemical parameters and clinical symptoms including dizziness and shortness of breath in pesticide workers in Pakistan compared with controls (unexposed males not involved in pesticide production) (Khan et al. 2010 cited in (NTP, 2020)). Other studies, such as those by Hyland et al. (2021), Mendy and Pinney (2022), Wu et al. (2024), Yang and Liang (2022), and more, investigated varied health impacts of neonicotinoid exposure including neurobehavioral outcomes, testosterone levels, obesity, hematologic parameters, and osteoporosis. Across these, common limitations highlighted were single spot samples for exposure assessment, lack of long-term exposure reflections, potential biases in lifestyle factors, and insufficient evidence to establish causality.

In conclusion, while findings indicate potential adverse impacts of neonicotinoid exposure on various health outcomes, including increased risks of ASD, congenital defects, liver enzyme alterations, and obesity, limitations such as reliance on self-reported data, categorical exposure estimations, short half-lives of neonicotinoids, and cross-sectional designs necessitate further longitudinal research to substantiate these associations.

The study designs and outcomes of the epidemiological studies, evaluating exposure to imidacloprid, and a general neonicotinoid exposure, are summarized in Table X.7 (Appendix – Toxicological profile).

In 2019, the Committee for Risk Assessment (RAC) of the ECHA evaluated human poisoning cases and concluded that they are not relevant for the classification of imidacloprid. The major drawback of available human data was that the exposure was not to pure imidacloprid but to insecticide formulations and that exposure levels could only be estimated roughly (RAC Opinion, 2019).

As no precise information on exposure is available in most epidemiological studies, the studies cannot be used to derive a guidance value.

5.2 Animal findings and in vitro studies

5.2.1 Toxicokinetics and metabolism

The metabolism of imidacloprid in animals was investigated in seven studies.

Female rats were orally exposed to a single dose of 20 mg/kg bw. The parent substance and two metabolites 6-chloronicotinic acid (6-CNA) and 6-hydroxy nicotinic acid (6-HNA) were detected in the brain, liver, kidneys and ovaries after 12 hours. In addition, bodily fluids (blood, urine) at 6, 12, 24 and 48 h and faeces at 24 and 48 h were measured with the maximum concentration Tmax 6 hours after exposure. The area under curve (AUC) of IMI ranged from 35 to 358 µg/mL/h; 6-CNA: 27.12 – 1006.42 µg/mL/h and 6-HNA: 14.98 – 302.74 µg/mL/h in different organs and bodily fluids. The clearance rate was maximum in ovaries followed by kidneys, liver, brain, faeces, blood and urine (Kapoor et al. 2014 as cited in (NTP, 2020)).

C^{14} labelled imidacloprid was given to rats (5/sex/dose) as a single dose of 1 mg/kg bw intravenously (i.v.) or as a single dose of 1 or 20 mg/kg bw orally. 48 h after treatment, the animals were sacrificed, and the concentration of total radiolabel was determined in organs and tissues. The substance was extensively absorbed and readily eliminated after oral exposure. After i.v. administration of 1 mg/kg bw about 92% of the recovered radiolabel was

excreted in urine and faeces within 48 h with most of the substance excreted via kidneys with an average ratio urine/faeces of 4:1 whereas 96% of the dose was excreted in urine and faeces within 48 hours after oral administration. More than 90% of renal radiolabel was excreted in the first 24 hours. The average residual radiolabel in the body, excluding the gastrointestinal (GI) tract, at sacrifice was about 0.5%, and 0.06% of the administered dose in the GI tract. There was no significant excretion in the exhaled air. The half-lives varied from 2.6-3.6 to 26-118 h (Klein 1987a as cited in (FAO and WHO, 2002)). Klein's study (Klein 1987a) also provides evidence of an enterohepatic circulation.

Imidacloprid D4 (imidazolidin-4,4,5,5 D4) was administered orally to male and female rats at a dose of 1 mg/kg bw and additionally to male rats at a dose of 150 mg/kg bw. The stable isotope labelled substance was determined in excreta and in plasma as a function of time. The administered stable isotope was extensively absorbed from the intestinal lumen and rapidly distributed in the body. Excretion was rapid, and the renal route predominated (Klein & Brauner 1991 as cited in (FAO and WHO, 2002)).

Four male mice were daily exposed to 0.6 mg/kg bw/d of IMI in the diet for 24 weeks. Brain, testis, lung, kidney, inguinal white adipose tissue and gonadal white adipose tissue mainly accumulated IMI, blood and mesenteric white adipose tissue mainly accumulated IMI-olefin; liver mainly accumulated desnitro-IMI; pancreas predominately accumulated 4-hydroxy-IMI. The desnitro-dehydro-IMI and the desnitro-IMI metabolites recorded tissue-blood concentration ratios ≥ 1.0 for testis, brain, lung and kidney (Nimako et al., 2021b).

Further information on toxicokinetic studies is available in Table IX.75 of the Appendix. The literature summarized in the Appendix serves to complement the toxicological profile of imidacloprid. Since human toxicokinetic are available which can be used for the HBM-GV calculation, animal data are included solely to provide a complete overview.

5.2.2 Acute toxicity with single administration

The acute toxicity of imidacloprid was investigated in several animal studies with rats and mice via the oral, dermal and inhalation route of exposure. Studies are summarised in Table X.9 of the Appendix.

EFSA considered imidacloprid as moderately acutely toxic in rodents with oral LD_{50} values of 380-650 mg/kg bw in rats and 130-170 mg/kg bw in mice. The dermal and inhalation acute toxicity is considered to be low with LD50 values of greater than 5000 mg/kg bw/d and greater than 5.32 mg/L, respectively (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013), see also table X.9.

In 2019, RAC evaluated the acute toxicity of imidacloprid. It was concluded that there are clear signs of acute toxicity via the oral route. While the rat data would support a classification in Category 4 according to CLP, the LD_{50} of 131 mg/kg bw in the mouse study supports Acute Tox 3 according to CLP (RAC Opinion, 2019).

5.2.3 Subacute, sub-chronic and chronic toxicity

Imidacloprid has been investigated in a large number of studies (specifically 43, 23 of which were published since 2020) with repeated exposure in mice, rats, rabbits and dogs with oral exposure via the diet or by gavage, inhalation, or dermal exposure. Studies are summarised in Table X.950 (Appendix). Most of the studies were not conducted under GLP conditions or did not follow any test guideline, only one dose was tested, or very specific endpoints were analysed. Thus, they are summarised only in the Appendix for complete documentary of the current literature related to the repeated dose toxicity.

The most reliable studies with sufficient exposure duration, study scope and OECD guideline conformity are described as follows.

Subacute

Short-term studies of oral and inhalation toxicity in mice, rats, and dogs and a study of dermal exposure in rabbits were conducted using imidacloprid. There was no consistent pattern of similar adverse effects in these studies. However, beagle dogs (2/sex/dose) being exposed up to 49 mg/kg bw/d in the diet for 28 days showed severe signs of toxicity for all animals from the high dose group (49 mg/kg bw/d) having died or being killed in extremis and moribund condition with ataxia, tremor, occasional vomiting, and markedly decreased body weight in these animals. Food consumption was decreased in animals of the mid (31 mg/kg bw/d) and high (49 mg/kg bw/d) dose

group. The NOAEL was 7.3 mg/kg bw/d (Bloch et al. 1987 as cited in FAO and WHO, 2002). Rabbits being dermally exposed for 15 days according to OECD test guideline 410 showed no adverse effects with a NOAEL of 1000 mg/kg bw/d (Flucke 1990 as cited in (FAO and WHO, 2002)). Rats being orally exposed to 0 or 45 mg/kg bw/d for 28 days showed extensive histopathological alterations in liver and kidneys. The LOAEL was defined at 45 mg/kg bw/d (Hassanen et al., 2022). Adverse hepatic effects were also observed in rats being orally exposed to a single dose of 25 mg/kg bw/d for 30 days with a LOAEL of 25 mg/kg bw/d (Askri et al., 2022). However, this study and further published after 2020 had several shortcomings like only a single dose group, small group sizes, very limited effect evaluations or being poorly described or not performed according to existing guidelines. Nonetheless, the study results may add weight to the evidence for hepatotoxicity, as they predominantly found effects related to this endpoint.An overview of the existing studies and results is available in Table X.95 of the Appendix.

Sub-chronic

In a repeated dose toxicity study according to OECD test guideline 408 and in compliance with GLP, 10 rats/sex/dose were exposed for 96 days to 0, 150, 600, 2400 ppm in the diet, corresponding to 0, 14.0/20.3, 60.9/83.3, 300.2/422.2 mg/kg bw/d for males and females, respectively. The NOAEL was 150 ppm (14.0 mg/kg bw/d) in males and 600 ppm (83.3 mg/kg bw/d) in females due to decreased bw and bw gain in males of the mid and high dose group and females of the high dose group. Slightly increased thromboplastin time as well as changes in clinical chemistry parameters were observed in high dose animals. Changes in organ weights were also observed in high dose animals as well as histopathological effects in the liver of males in the mid and high dose but these effects were reversible (Eiben and Rinke 1989 as cited in FAO and WHO, 2002).

Beagle dogs (4/sex/dose) were exposed for 90 days to 0, 200, 600, 1800/1200 ppm in the diet, corresponding to 0, 7.8, 23.0, 42.0 mg/kg bw/d for males and females according to OECD test guideline 409 and in compliance with GLP. The high dose group of 1800 ppm was decreased to 1200 ppm after 4 weeks due to low food consumption. Trembling occurred in all animals from 600 ppm and severe trembling occurred at 1800 ppm, but no such effect was observed after reduction to 1200 ppm. Body weight gain was decreased at 1800 ppm, but was normal after reduction to 1200 ppm. Food consumption was decreased in the high dose group at 1800 and 1200 ppm and to a lesser extent at 600 ppm. Males at 600 ppm showed ophthalmologically transient slight corneal erosion. Slight haematological, clinical chemistry and urinalysis changes were independent of dosage and changes in organ weights were within historical control data. There were no histopathological findings. The NOAEL was 200 ppm (7.8 mg/kg bw/d) for males and females (Ruf 1990 as cited in (FAO and WHO, 2002)).

Mice and rats (each 10/sex/dose) were exposed to 0, 120, 600 or 3000 ppm, corresponding to 0, 17, 86, 430 mg/kg bw/d for mice and 0, 11, 57, 410 mg/kg bw/d for male rats and 15, 78, 510 mg/kg bw/ for female rats through the diet for 107 and 98 days, respectively. In mice of the high dose group, 7 males and females died and there was poor general condition in several animals. Decreased body weight gain and food consumption was observed in the males of the mid and high dose group and females of the high dose group. Organ weight of high dose animals were severely affected as well as clinical chemistry parameters.

In rats, body weight gain was decreased as well in mid and high dose group animals, and food intake was decreased in the high dose group. Clinical chemistry was affected in the high dose group and there were degenerative histological lesions in the epithelium of the testicular tubules and multifocal group cell necrosis in liver of high dose (HD) males. The NOAELs were 120 ppm, corresponding to 17 mg/kg bw/d in mice and 11 mg/kg bw/d in rats (Eiben 1998a and b as cited in (FAO and WHO, 2002)). Further studies and results are available in TableTable X.95. Further newer studies published after 2020 had several shortcomings like only a single dose group, small group sizes, very limited effect evaluations or being poorly described or not performed according to existing guidelines and are therefore not suitable for deriving a threshold value. Nevertheless, some study results can lend additional weight to the results of the guideline studies.

Chronic

4 Beagle dogs/sex/group were exposed in a chronic toxicity study according to OECD test guideline 452 and in compliance with GLP for 52 weeks to 0, 200, 500, 1250/2500 ppm in the diet, corresponding to 0, 6.1, 15, 41/72 mg/kg bw/d for males and females, respectively. The highest dose was increased from week 17 onwards from 1250 to 2500 ppm (or from 41 to72 mg/kg bw/d). There was no increased mortality, but food consumption was

decreased in high dose animals in the first week and after increasing the concentration. Clinical chemistry parameters like cholesterol were significantly increased in females of the high dose group, liver cytochrome levels were significantly increased in high dose males and females. Relative brain weight was slightly increased in high dose animals but was considered as adaptive process due to induced metabolism. There were no gross pathological or histopathological findings. The NOAEL was 500 ppm, equal to 15 mg/kg bw/d (Allen et al. 1989 as cited in (FAO and WHO, 2002)).

In a combined chronic toxicity/carcinogenicity study according to OECD test guideline 453 and in compliance with GLP (see also chapter 5.2.7), 50 Wistar rats/sex/dose were exposed for two-years to 0, 100, 300, 900, 1800 ppm in the diet, corresponding to 5.7/7.6, 16.9/24.9, 51.3/73.0, 102.6/143.7 mg/kg bw/d for males and females, respectively. Body weight gain was decreased dose dependently at 900 and 1800 ppm and water intake was decreased in high dose animals. Aspartate amino transferase was significantly increased in the high dose group, creatine kinase levels were increased in females of the high dose group only. Decreased absolute and relative organ weights in the high dose were considered secondary to decreased body weight. There were treatment-related lesions in the thyroid gland: there was a marked, dose-dependent increase in the incidence and severity of mineralized particles in the thyroid follicles. This effect became statistically significant in male rats fed 300 ppm of imidacloprid and in females at 900 ppm ($p \leq 0.001$). In general, the severity of occurrence in control rats appeared to be minimal (Grade1). In view of the historical data, the 100 ppm dose-group males (28% mineralized particles) was in the historical range. The NOAEL from this study was 100 ppm (5.7 mg/kg bw/day), based on an increase in incidence and severity of mineralized particles in thyroid gland in male rats at the LOAEL of 300 ppm (17 mg/kg/day). However, the plasma levels of thyreotropin, triiodothyronine and thyroxine were unaffected. (Eiben, 1991 and Eiben and Kaliner, 1991).

Fifty B6C3F1 mice/sex/dose were exposed for 24 months in a carcinogenicity study according to OECD test guideline 451 and in compliance with GLP to 0, 100, 330, 1000, 2000 ppm in the diet, corresponding to 0, 20.2/30.3, 65.6/103.6, 208.2/274.4, 413.5/423.9 mg/kg bw/d for males and females, respectively (see also chapter 5.2.7). There was no treatment related increased mortality. Body weight and body weight gain was decreased in males of the mid dose and significantly decreased in high dose males. Food consumption was decreased in mid dose females, and was significantly so in high dose females. Water intake was decreased dose dependently in all animals from the mid and high dose group. There were depressed leukocyte counts in HD animals, increased alkaline phosphatase (AP) and decreased cholesterol in HD animals. Absolute lung, liver, kidney, adrenals, brain, ovary weights were significantly decreased. Gross pathological findings such as discolorations and changes in size and shape of several organs, cystic and enlarged ovaries, collapsed lungs, hair loss in skin and nodes were observed in several organs. There was a slight periacinary liver cell hypertrophy and loss of vacuolation in the tubules of kidneys in HD males and an increased incidence of mineralization in the brain (thalamus) in HD animals. Greatly decreased incidences of tubular basophilia in HD males and of lymphocytosis in the renal pelvis in HD males and females might be secondary to decreased food consumption. The NOAEL was determined at 330 ppm, equal to 65.6 and 103.6 mg/kg bw/d in males and females, respectively (Watta-Gebert, 1991a,b as cited in (FAO and WHO, 2002)). Further details are available in Table X.99. No further chronic studies are available.

The lowest NOAEL was determined in the combined chronic toxicity/carcinogenicity study according to OECD test guideline 453 with a value of 5.7 mg/kg bw/d. The critical effect was decreased body weight gain, changes in clinical chemistry parameters and a dose dependent thyroid mineralisation (mineralisation in the follicular colloid). This NOAEL was used by EFSA to derive the ADI with an assessment factor of 100 to a rounded value of 0.06 mg/kg bw/d.

5.2.4 Neurotoxicity

There are numerous studies available (specifically 21, of which 7 were published since 2020) in the field of neurotoxicity of imidacloprid in animals.

"No specific neurotoxic effects were observed in the 13-wk neurotoxicity study in rats (note: see also below Sheets 1994b). In the developmental neurotoxicity study in rats, direct (but no developmental) neurotoxic effects on the pups were noted at maternal dose levels of 80.4 mg/kg bw/d and higher, the NOAEL being 45.4 mg/kg bw/d." (https://dissemination.echa.europa.eu/Biocides/ActiveSubstances/0037-18/0037-18_Assessment_Report.pdf, cf. page 15)

In functional observational batteries (FOB) in rats, dose dependent effects like tremor, uncoordinated gait, decreased activity in combination with signs of general toxicity were observed after single exposure to 0, 42, 150 and 350 mg/kg bw/d via gavage. The NOAEL was 42 mg/kg bw/d (Sheets 1994a as cited in FAO (FAO and WHO, 2002)). A dose dependent increased incidence of tremors, uncoordinated gate and decreased activity was also observed in rats being exposed to single doses of 0, 20, 50, 150 and 350 mg/kg bw/d with a NOAEL of 50 mg/kg bw/d (Anonymous 1992 as cited in ECHA registration dossier). Rats being orally exposed to 0, 150, 1000, 3000 ppm, corresponding to 0, 10.5/9.3, 69.5/63.3, 213/196 mg/kg bw/d for males and females, respectively over a period of 90 days showed slightly uncoordinated grip strength and slightly increased motor and locomotor activity in high dose males and lower motor and locomotor activity in females, but these observations were considered incidental due to lack of significance (Sheets 1994b as cited in (FAO and WHO, 2002)).

Anxiety-like behaviour and locomotor activity using the open field test, depression-like behaviour using the forced swim test were observed in mice being exposed to 0.5 and 1 mg/kg bw/d over a period of 60 days (Khali 2017 as cited in (Ongono et al., 2020a)). Another study (Tonietto et al. 2022) on adult male rats exposed to oral doses of 0, 1.5, 5, and 15 mg/kg bw/day for 45 days noted increased rearing (15 mg/kg bw/day group) and time spent at the periphery in locomotor activity tests (5 mg/kg bw/day group), reduced time to complete the OX maze task (5 mg/kg bw/day group), decreased mean corpuscular haemoglobin concentration (15 mg/kg bw/day group), and increased serum butyrylcholinesterase activity (15 mg/kg bw/day group).

Significantly decreased learning skills and memory consolidation was observed in rats being exposed to 2 and 8 mg/kg bw/d in the diet for 90 days. In pups being exposed from gestation day 0 to lactation day 21, decreased motor activity was observed but these effects were transient (Kara 2015).

The other studies demonstrate adverse neurotoxic effects and oxidative stress linked to imidacloprid exposure (partly in concentrations lower than the EFSA POD but in experimental settings with only one concentration). In the study by Abdelhafez et al., 2023 adult male rats were gavaged with 0, 22, or 45 mg/kg bw/day of imidacloprid over 28 days to study its effects on the central nervous system. Significant reductions in acetylcholine esterase activities and elevations in malondialdehyde concentrations indicated oxidative stress.

Additional studies and further details are summarized in Table IX.77 of the Appendix. Most of the studies were not conducted under GLP conditions, did not follow any test guideline, or only one dose was tested so that no dose-response relationship can be determined. The results of these studies are only considered to be supporting evidence.

5.2.5 Effects on skin and mucous membranes

There is one acute skin irritation/corrosion study available in New Zealand White rabbits exposed following the OECD test guideline 404, one acute eye irritation/corrosion study in New Zealand White rabbits exposed following the OECD test guideline 405 and one skin sensitisation study in male Dunkin/Hartley guinea pigs exposed following the OECD test guideline 406. All the three studies were negative. It is therefore concluded that imidacloprid has no skin sensitising or irritation potential. The studies are summarised in Table 79 (Appendix).

5.2.6 Genetic toxicity

The genotoxicity of imidacloprid has been investigated in a comprehensive range of *in vitro* and *in vivo* assays. The experimental protocol included five negative *Salmonella* reverse mutation assays, conducted with and without metabolic activation, in accordance with OECD test guideline 471, using concentrations up to 12,000 µg/plate. Additionally, a negative mammalian cell micronucleus test was performed using human peripheral blood lymphocytes, in accordance with OECD test guideline 487. Furthermore, a negative mammalian cell gene mutation assay was performed in CHO cells following OECD test guideline 476. Further assays also included a negative DNA damage assay, a negative mitotic recombination assay, and unscheduled DNA synthesis and sister chromatid exchange in CHO cells. The only positive result in an *in vitro* test was a chromosome aberration study in human lymphocytes following OECD test guideline 473 which induced questionable clastogenic effects according to (FAO and WHO, 2002).

Available *in vivo* tests were four mammalian erythrocyte micronucleus tests with three of them being performed following the OECD test guideline 474 with concentrations up to 2000 mg/kg bw/d, a mammalian spermatogonial

chromosomal aberration test in mice following the OECD test guideline 483, a negative mammalian bone marrow chromosomal aberration test following the OECD test guideline 475 up to 2000 mg/kg bw and a negative chromatid exchange up to 2000 mg/kg bw/d. A positive result gave a single modified bone marrow chromosomal aberration test with dose dependent effects but the exposure in this test was not clear and according to the authors, the positive effect could be attributed to a synergistic toxic effect from the multiple chemical exposure (imidacloprid and methamidophos) as well as the prolonged exposure to the two doses of the insecticides (Karabay and Oguz, 2005).

Further studies and additional information are summarised in Table X.98. Only two studies published since 2020 are available, both investigating the protective effect of olive oil on the mutagenic effects of imidacloprid (Mahmoud et al., 2023; Mohamed et al., 2020).

In summary, the negative test results far outweigh the positive ones. The positive results of the *in vitro* and *in vivo* tests are questionable (see above mentioned arguments). In a weight of evidence approach it can be concluded that imidacloprid is not genotoxic. This is also confirmed by (FAO and WHO, 2002).

5.2.7 Carcinogenicity

Chronic toxicity data for imidacloprid are available from two exposure studies conducted in rats and mice via the oral route. No chronic exposure data on imidacloprid are available for humans or primates. Studies are summarised in Table X.99 of the Appendix.

In a 24-month GLP compliant combined chronic toxicity/carcinogenicity study following the OECD test guideline 453, 50 Wistar rats/sex/dose were exposed to dietary concentrations of 0, 100, 300, 900, 1800 ppm, corresponding to 0, 5.7/7.6, 16.9/24.9, 51.3/73.0, 102.6/143.7 mg/kg bw/d for males and females, respectively. Survival was not affected and there was no treatment related neoplastic effects observed in the study. The total number of tumours observed and the incidence of benign and malignant neoplasms were comparable in all study groups with most tumours being observed in the pituitary gland, thyroid, mamma and uterus. An increased incidence of liver tumours in males of the high dose group (one adenoma, one carcinoma and two cholangiocellular carcinomas) were within the range of historical control data and are therefore not considered treatment related (Eiben, 1991 and Eiben and Kaliner, 1991 as cited in (FAO and WHO, 2002)).

In the second GLP compliant carcinogenicity study in accordance with the OECD test guideline 451, 50 B6C3F1 mice/sex/dose were exposed for a period of two years to dietary concentrations of 0, 100, 330, 1000 or 2000 ppm, corresponding to 0, 20.2/30.3, 65.6/103.6, 208.2/274.4 or 413.5/423.9 mg/kg bw/d for males and females, respectively. Survival was again not affected. An increased incidence of low-grade periacinar hepatic-cell hypertrophy was found in males at 2000 ppm. There was no evidence of a carcinogenic effect. The spectrum of neoplastic lesions in control animals was consistent with that expected in ageing mice with no evidence of a treatment-related increase in the incidence of tumour-bearing animals or of specific tumour types and there were no proliferative lesions in any treatment group of either sex that were considered to be definitively test article related (Watta-Gebert 1991a,b as cited in (FAO and WHO, 2012)).

According to (FAO and WHO, 2002) there was no evidence of an carcinogenic effect of imidacloprid in mice and rats. Our recent review of the current data from the literature search on the carcinogenic potential of imidacloprid did not reveal any new or additional findings and no additional studies dealing with the carcinogenicity of imidacloprid were found.

5.2.8 Reproductive toxicity

Effects on fertility

There are numerous studies available for imidacloprid with data on fertility effects in animals (specifically 14, of which 9 were published since 2020). An overview on existing studies dealing with effects on fertility in animals can be found in Table IX.82 in the Appendix. Many of these studies were not GLP-compliant or did not follow any test guideline, as very specific endpoints were investigated. Imidacloprid was also only tested in one single dose in the majority of cases. Studies that meet GLP standards, with adequate long-term exposure according to OECD test guidelines and a range of doses, are described below.

The most important study is a two-generation reproduction toxicity study following the OECD test guideline 416 and in compliance with GLP. This study used 30 Wistar rats/sex/dose in F_0 and 26/sex/dose in F_1 at concentrations of 0, 100, 250 or 700 ppm in the diet, corresponding to 0, 6.6, 17 and 47 mg/kg bw/d, for 84 days before mating and throughout mating, gestation and lactation of the F_{1a} and F_{1b} litters. The exposure started 105 days before breeding of the F_{2a} and F_{2b} litters. In high dose (47 mg/kg bw/d) F_0 males, decreased food consumption and body weight gain were observed. Decreased food consumption was also observed in F_0 and F_1 females and reduced body-weight gain in F_0 females. Both effects were observed in all generations (F_{1a}, F_{1b}, F_{2a} and F_{2b}) at 700 ppm (or 47 mg/kg bw/d) during lactation. In P_0, the mating performance, fertility, duration of gestation and litter size were normal but one high dose female lost its entire F_{1b} litter between day 6 and 12 of lactation period which was considered incidental. In P_1, no evidence of an adverse effect of treatment was observed on mean precoital time, fertility index, conception rate and gestation index. However, at 250 ppm, a significantly increased breeding loss in F_{2a} litters from day 4-21 postpartum was observed but within normal range of this strain and therefore considered incidental. Decreased absolute liver weights in high dose F_{1a} and F_{1b} male and female pups and decreased absolute testes weight in F_{1a} pups and decreased relative testes weight increased in F_{1b} pups considered incidental since in no other generation these effects have been observed. An increased absolute and relative ovary weight in F_{2a} litters of the mid dose was without dose dependency and therefore not considered treatment related. According to the registration dossier, the NOAEL for reproductive effects was 700 ppm (or 47 mg/kg bw/d), the highest concentration tested.

On the other hand, decreased sperm morphology and concentrations were observed after male mature and immature groups were exposed to imidacloprid via gavage for 65 days (Mohammed et al. 2017 as cited in (NTP, 2020)), after being exposed to doses up to 2.8 mg/kg bw/d for 90 days (Bal 2012c as cited in (NTP, 2020)) and following oral gavage to 9 mg/kg bw/d for one month in male albino rats (Abdel-Razik et al., 2021).

There is a recent publication, in which Wistar rats were exposed orally to imidacloprid at the ADI level of 0.06 and 0.6 mg/kg bw/d for a period of 90 days. In this study, imidacloprid caused abnormalities in sperm concentrations and morphologies, accompanied by an imbalance of the gonadal hormone testosterone but these effects were observed without any dose dependency. Histopathological damage and a decrease of testosterone levels were observed in testes from treated rats but these effects occurred again without any dose dependency (G. Zhao et al., 2021).

Further minor effects on male reproductive organs and sperm parameters were observed in additional studies as it is summarized in Table IX.82 of the Appendix. However, the severeness of these effects is not clear from the descriptions. In most cases there was only a single dose group with small group sizes, very limited effect evaluations or being poorly described or not performed according to existing guidelines and are therefore not suitable for deriving a threshold value.

According to the ECHA Brief Profile (last update 20/03/2025), imidachloprid is under evaluation in terms of possible endocrine disrupting properties.

Developmental toxicity

The developmental toxic effects of imidacloprid have been investigated in several studies (specifically 9, of which 3 were published since 2020) which are summarised in Table of the Appendix.

The most important studies in terms of duration of exposure and scope of investigation were two prenatal developmental toxicity studies in rats and rabbits following the OECD test guideline 414 and in compliance with GLP.

In the first study, Wistar rats (25 dams/dose) were exposed via gavage to 0, 10, 30, 100 mg/kg bw/d from gestation day (GD) 0-15. The animals were sacrificed on GD 21. There were signs of maternal toxicity like decreased body weight gain in the mid dose which was significant in the high dose and decreased food consumption in all treated animals which was increased in the high dose group after end of treatment. In foetuses of treated animals, a change in sex ratio was observed with a male to female ratio of 58.6/41.4% compared to 51.0/49.0% in the control group. However, this was not observed in a dose range finding study at an even higher dose of 150 mg/kg bw/d and at 100 mg/kg bw/d the ratio was shifted in favour of females. Therefore, this effect was considered incidental. In HD foetuses, wavy ribs were slightly more frequently and visceral malformations in form of severe haemorrhage

around the brain and dilated lateral ventricles of the brain occurred. In addition, enlarged hearts (auricles were dilated with blood and the walls of the ventricles were thickened) occurred. However, the effects were considered secondary to maternal toxicity and wavy ribs are a variation rather than a malformation, and therefore not considered adverse. The NOAEL for maternal effects was 10 mg/kg bw/d and the NOAEL for developmental effects was 30 mg/kg bw/d (Becker et al. 1988a as cited in (FAO and WHO, 2002)).

In the second study, Chinchilla rabbits (16 dams/dose) were exposed by gavage to 0, 8, 24 or 72 mg/kg bw/d from GD 6-18 and were sacrificed on GD 28. Two females of the high dose group died on GD 18 and 19, body weight loss and decreased food consumption in mid and high dose groups were additional signs of maternal toxicity. In one female of the high dose group abortions occurred, and slightly increased post implantation losses in several HD females were observed with total litter resorption in two of them. Foetal body weight was significantly decreased in the high dose group. Due to abortion and post implantation loss the live foetuses from the high dose group decreased. There was an increased incidence of non-ossified phalangeal nuclei and metacarpalia of both fore limbs and phalangeal nuclei of both hind limbs, but these effects were considered secondary to maternal toxicity. The NOAEL for maternal toxicity was 8 mg/kg bw/d and the NOAEL for developmental toxicity 24 mg/kg bw/d (Becker et al. 1988b as cited in (FAO and WHO, 2002)).

Further recent studies are available. They are not following any guideline, had very small group sizes and only one dose group. In one recent study 10 male neonatal rats per dose were exposed to 0, 1, 5 or 10 mg/kg bw/d with histopathological changes in the testis and epididymis, decreased plasma concentrations and increased ROS production. However, the route of exposure is not clear from the description and there is no clear dose dependency in the effects so that a NOAEL cannot be derived from the study (Sardar et al., 2023).

5.2.9 Immunotoxicity

Only very limited data is available on the immunotoxicity of Imidacloprid. In a single study investigating the immunotoxicity of imidacloprid following the EPA OPPTS 870.7800 and in compliance with GLP, 10 male Wistar rats per dose were exposed to 0, 150, 600 or 2400 ppm, corresponding to 0, 11.7, 47.1, 186 mg/kg bw/d in the diet for 28 days. There was no increased mortality, although one male of the high dose group showed signs of general toxicity like piloerection and wasted appearance. Body weight and body weight gain was decreased in high dose animals as well. However, there were no immunological findings in this study. A decrease of absolute thymus weight and relative spleen weight was considered secondary to body weight losses and was within the range of historical control data. Atrophic and small thymus in the high dose group (3/10) and congested/red intestine (3/10), were also considered secondary to general toxicity. The NOAEL for immunotoxicity was 186 mg/kg bw/d, the highest dose tested. The overall NOAEL for the test substance in this study was 47.1 mg/kg bw/d (Anonymous 2010 as cited in ECHA registration dossier), based on systemic effects of decreased body weight and food intake, as well as secondary impacts on thymus and spleen weight and some localized tissue changes.

5.2.10 Other findings

In a recent in vitro study, the effects of Imidacloprid on human cells were investigated. In this study, the mitochondria function, oxidative stress, DNA damages, and genes transcription levels were examined in the human neuroblastoma SH-SY5Y cells after 48-h exposure to imidacloprid at concentrations from 0.05 to 200 µmol/L. It was demonstrated that imidacloprid caused mitochondrial dysfunction by degrading the levels of mitochondrial membrane potential (MMP) and adenosine triphosphate (ATP). Furthermore, by disrupting calcium ion levels and mitochondrial activity, imidacloprid promoted the production of reactive oxygen species (ROS) and hydrogen peroxide, which in turn led to oxidative stress. In the end, at imidacloprid concentrations greater than 47.6 µmol/L, the oxidative stress still caused DNA damage and death in SH-SY5Y cells. With a median activity value of 0.74 µmol/L, ATP was the most sensitive endpoint among those studied (Wei et al., 2024). Oxidative stress is one of the early markers in several AOPs.

6 Derivation of human biomonitoring values

According to (Apel et al., 2020), human biomonitoring (HBM) studies allow to identify and quantify chemicals of concern or their metabolites in human biological material and, using HBM-GVs, a health-based interpretation of the measurements to determine whether (further) chemical management or regulatory action is required.

Existing data on imidacloprid allows for the derivation of HBM-guidance values (HBM-GVs) for the general population that correspond to internal exposure concentrations at which there is no appreciable risk.

6.1 Choice of the critical effect and POD

The choice of a critical effect should be based on chronic exposure. Two chronic toxicity/carcinogenicity studies were conducted in rats and mice with an exposure duration of 24 months (following the OECD test guideline 453 and 451). The results indicated no increase in tumor incidences across both species. Consequently, no critical effects to carcinogenicity that may be considered.

However, non-neoplastic lesions occurred in rats after oral exposure with decreased body weight gain, changes in clinical chemistry parameters and a dose dependent thyroid mineralisation observed at 300 ppm and above, leading to a NOAEL of 100 ppm, corresponding to 5.7 mg/kg bw/d (Eiben, 1991 and Eiben and Kaliner, 1991 as cited in (FAO and WHO, 2002) and referred to by EFSA, 2013) as described in section Subacute, sub-chronic and chronic toxicity. This NOAEL of 5.7 mg/kg bw/d is the lowest NOAEL for imidacloprid established in a reliable study following adequate test guidelines.

Other studies that were considered reliable such as those by (Allen et al. 1989 or Watta-Gebert, 1991a,b as cited in (FAO and WHO, 2002)) revealed similar or higher NOAELs when compared to the studies of (Eiben, 1991 and Eiben and Kaliner, 1991 as cited in (FAO and WHO, 2002).

According to (Apel et al., 2020), there are three options to derive the HBM-GV. The first and preferred option is to choose the POD according to a critical effect from human data, the second best option is to use a defined external TRV, proposed by a European or relevant non-European institution and the third option is to use a POD based on a critical effect observed in experimental animal studies. Since no adequate human data is available, the second option was considered. Since the current data, based on the literature review, did not provide any indications to revise EFSA's existing ADI, the ADI presented in Table X.3 is used[65]. This value is preferred over others because it is based on a chronic toxicity/carcinogenicity study conducted in accordance with OECD 453 and following the GLP standards, and its NOAEL is the lowest among the reliable values identified in the current literature review.

The EFSA ADI of 0.06 mg/kg bw/d for imidacloprid dates back to 2008 and was confirmed by the EFSA scientific opinion (EFSA, 2013). This ADI was based on studies by Eiben (1991) and Eiben and Kaliner (1991), with a NOAEL of 5.7 mg/kg bw/d for critical effects including decreased body weight gain, changes in clinical chemistry parameters and a dose dependent thyroid mineralization. After applying the standard uncertainty factor of 100, this NOAEL of 5.7 mg/kg bw/d led to the rounded ADI.

[65] For information only: The subchronic Non-Cancer Health Risk Limit (nHRLSubchronic) = nHRLShort-term = 2 µg/L for drinking water of the Minnesota Department of Health (MDH) (https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/imidasumm.pdf) is based on the POD value of 0.820 mg/kg bw/d, which is still higher than the POD used in our assessment.

6.2 Derivation of HBM-GVs

If no more differentiated PBPK models are available, an HBM-GV can be derived from a TRV as described in (Apel et al., 2020) by using the urinary mass balance approach knowing the rate of excretion of the active substance or its specific metabolite(s), the molecular weight of the parent substance and its metabolite(s) and the urinary flow rate related to the body mass.

The general formula for deriving an HBM-GV for one main metabolite is:

$$HBM - GV = TRV \times \frac{\left(\frac{MW(Metabolite) \times Fue(Metabolite)}{MW(Substance)}\right)}{Daily\ urinary\ flow\ rate}$$

For imidacloprid, IMI-olefin in urine was classified as specific and most suitable biomarker for human biomonitoring (Wrobel et al., 2023)[66] as described in section Toxicokinetics and metabolism.

For IMI-olefin, 27 ± 8% were found after 48 hours. This value of 27 % is used for the urinary mass balance approach. The resulting urinary excretion fraction (F_{ue}) is the decimal of the percentage i.e., 0.27 for IMI-olefin as inserted into the formula.

The molecular weight of the parent substance imidacloprid is 255.7 g/mol and that of its metabolite IMI-olefin is 253.7 g/mol. The currently used body weight proportional urine volume per day enters the calculation with 0.03 L/kg bw/day for children and men and 0.035 L/kg bw/d for women (German HBM Commission, 2025, submitted).

Using the EFSA TDI of 0.06 mg/kg bw/d from 2013, the urinary mass balance approach for the major specific metabolite IMI-olefin is for children and men:

$$\boldsymbol{HBM - GV = \frac{0.06\frac{mg}{kg}bw}{d} \times \frac{\left(\frac{253.7\frac{g}{mol} \times 0.27}{255.7\frac{g}{mol}}\right)}{\frac{30\frac{mL}{kg}bw}{d}} = 0.00054\frac{mg}{mL} = 0.54\ mg/L}$$

and for women:

$$\boldsymbol{HBM - GV = \frac{0.06\frac{mg}{kg}bw}{d} \times \frac{\left(\frac{253.7\frac{g}{mol} \times 0.27}{255.7\frac{g}{mol}}\right)}{\frac{35\frac{mL}{kg}bw}{d}} = 0.00046\frac{mg}{mL} = 0.46\ mg/L}$$

Thus, the HBM-GV for IMI-olefin is 0.54 mg/L urine for children and men and 0.46 mg/L urine for women.

The derived HBM-GVs represent the concentration of IMI-olefin in human urine up to which – for single substance evaluation – a risk for adverse health effects is not anticipated.

When assessing HBM results, it must be taken into account that exposure to mixtures of substances is usually present, so that the additional use of a MAF (mixture assessment factor/mixture allocation factor) or another suitable methodology should be considered for further assessments. At least structurally related substances or substances that display the same effect targets should be considered together for the next review and refinement of the HBM-GV.

The HBM-GVs are preferably given in relation to volume and not in relation to creatinine, as the physiological creatinine formation in healthy adults varies according to gender and is significantly reduced with age. Creatinine formation in children is also significantly lower and, moreover, strongly age-dependent. Meat consumption also contributes as an external factor to the creatinine pool. These aspects should be considered when adjusting

[66] Hydroxylated metabolites 4-/5-OH-IMI in urine were also considered good and robust biomarkers of exposure in urine. However, according to (Wrobel et al., 2023), the analytical method used in the study quoted does not separate both isomers and the detector response for these two analytes might differ to some extent. This means that the application of the reported Fue for reverse dosimetry would require, strictly speaking, the specific use of the "Wrobel, 2023 method" and can therefore not be used for a general application.

biomarker measurements in urine for creatinine (Carrieri et al., 2001; Commission Human Biomonitoring of the German Environment Agency, 2005; Sallsten and Barregard, 2021; Abraham et al., 2023).

For better clarity, the following Table X.91 presents a summary of key parameter values along with the resulting HBM-GVs values.

Table X.91: Summary of parameter values used for the HBM-GV calculation

$HBM\text{-}GV_{Children\ and\ men}$ for the specific metabolite IMI-olefin in urine	0.54 mg/L
$HBM\text{-}GV_{Women}$ for the specific metabolite IMI-olefin in urine	0.46 mg/L
$Molar\ mass_{IMI\text{-}olefin}$	253.7 g/mol
$Fue_{IMI\text{-}olefin}$	0.27
ADI	0.06 mg/kg bw/d

It should be taken into account that due to the short half-life of excretion of IMI-olefin more reliable evaluations of the extent of exposure could be made based on 24h urine collections rather than spot samples. Moreover, a publication by Casas et al. (2018) quantifying the variability of biomarker measurements of many non-persistent chemicals during several time windows shows that for many of these compounds a few dozen samples are required to accurately assess exposure over periods encompassing several trimesters or months. Exposure misclassification in epidemiological studies is expected to be high.

On the other hand, on a population basis, spot and 24-h samples produce rather comparable results, e.g. see: Christensen et al. (2012) "Overall, spot urinary concentrations of DEHP metabolites and BPA have variability roughly comparable with corresponding 24-h average concentrations obtained from a comparable population, suggesting that spot samples can be used to characterise population distributions of intakes. However, the analysis also suggests that caution should be exercised when interpreting the high end of spot sample data sets."

6.3 Level of confidence attributed to the derived HBM guidance values

As mentioned in the HBM4EU concept document to derive HBM-GVs (Apel et al., 2020), it is suggested to attribute a level of confidence (low, medium or high) to each calculated HBM-GV. The level of confidence should reflect the uncertainties identified during the elaboration of the value and could constitute a good incentive to later revise values with an estimated higher level of confidence.

The data used for calculation of the urinary excretion fraction was derived from a study with only three male volunteers, who received a single oral imidacloprid dose of 5 mg (Wrobel et al., 2023). As toxicokinetics are subject to considerable individual variability, it is difficult to draw general conclusions from this single measurement. The variability can depend in particular on the age, gender or ethnicity of the volunteer but can also vary in supposedly similar population groups. Hence, the data is not representative of the entire population. However, since the ADI used already includes a safety factor of 10 to cover intraspecies variability, it can be concluded that the variability that has occurred is already covered to some extent but imidacloprid specificities are not taken into account due to lack of data. Therefore, the level of confidence is **medium** for this aspect.

The ADI is based on a combined chronic toxicity/carcinogenicity study following the OECD test guideline 453 in rats and in compliance with GLP (Eiben, 1991 and Eiben and Kaliner, 1991 as cited in (FAO and WHO, 2002)) with a NOAEL of 5.7 mg/kg bw/d for decreased body weight gain, changes in clinical chemistry parameters and a dose dependent thyroid mineralisation. The same study was identified as the key study in the performed literature search for this project. Hence, the POD is considered suitable. Nevertheless, the key study for the derivation of the ADI is based on animal data. The best case for the derivation of HBM-GVs would have been availability of human data. The level of confidence for this aspect is therefore **medium**.

Developmental immunotoxicity and neurotoxicity are generally regarded as critical endpoints, However, the current ADI for imidacloprid is considered by EFSA to be sufficient to protect against its potential developmental neurotoxic effects. There have been no indications to question this assessment. Nevertheless, additional studies

regarding developmental immunotoxicity and neurotoxicity would be desirable. For this reason, a **low** level of confidence is awarded for this aspect.

Many studies could not be viewed in the original because they were company studies. Therefore, the level of confidence for this aspect is **low**. Nevertheless, the assessment was based on critical evaluations previously done by (mainly) authorities so that it can be assumed that a reliable assessment was carried out.

The overall level of confidence of HBM-GVs for imidacloprid is considered to be low-medium.

It should be noted, though, that the levels of confidence suggested in this document are based on expert judgement regarding the accuracy of the data and the process of calculation used to derive the HBM guidance values, rather than being established in accordance with a recognized and accepted methodology.

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8 Appendix – Toxicological profile

8.1 Search Terms

PubMed

(("Imidacloprid"[Title/Abstract] OR ("IMI"[Title/Abstract] OR "6-Chloronicotinic acid"[Title/Abstract]) OR "6-CNA"[Title/Abstract] OR "imidazolidine 4-"[Title/Abstract] OR ("imidazolidine 5-"[Title/Abstract] OR "olefinic imidacloprid"[Title/Abstract]) OR "desnitro imidacloprid"[Title/Abstract] OR "hydroxy imidacloprid"[Title/Abstract]) AND ("health"[Title/Abstract] OR "adverse"[Title/Abstract] OR "toxic*"[Title/Abstract] OR "neurotox*"[Title/Abstract] OR "expos*"[Title/Abstract] OR "epidem*"[Title/Abstract] OR "cohort"[Title/Abstract] OR "genotox*"[Title/Abstract] OR "genetic*"[Title/Abstract] OR "carc*"[Title/Abstract] OR "cancer"[Title/Abstract] OR "reproduc*"[Title/Abstract] OR "reprotox*"[Title/Abstract] OR "developmental"[Title/Abstract] OR "fertil*"[Title/Abstract] OR "disease"[Title/Abstract] OR "neurologic*"[Title/Abstract] OR "metabol*"[Title/Abstract] OR "ADME"[Title/Abstract] OR "PBPK"[Title/Abstract] OR "PBTK"[Title/Abstract] OR "excret*"[Title/Abstract] OR "in vivo"[Title/Abstract] OR "in vitro"[Title/Abstract] OR "in silico"[Title/Abstract] OR "endocrine"[Title/Abstract] OR "half-life"[Title/Abstract] OR "immun*"[Title/Abstract] OR "MOA"[Title/Abstract] OR "mode of action"[Title/Abstract])) AND (2020:2024[pdat])

Scopus

(TITLE-ABS-KEY (imidacloprid OR {IMI} OR {6-Chloronicotinic acid} OR {6-CAN} OR {4-imidazolidine} OR {imidazolidine 4-} OR {imidazolidine 5-} OR {5-imidazolidine} OR { olefinic imidacloprid } OR {desnitro imidacloprid} OR {hydroxy imidacloprid}) AND TITLE-ABS-KEY (health OR adverse OR {mode of action} OR toxic* OR neurotox* OR epidem* OR cohort OR genotox* OR genetic* OR carc* OR cancer OR reproduc* OR reprotox* OR developmental OR fertil* OR disease OR neurologic* OR metabol* OR adme OR pbpk OR pbtk OR excret* OR {in vivo} OR {in vitro} OR {in silico} OR endocrine OR {half-life} OR immun* OR moa OR {mode of action})) AND PUBYEAR > 2019 AND PUBYEAR < 2025 AND (LIMIT-TO (LANGUAGE , "English")) AND (EXCLUDE (EXACTKEYWORD , "Insecticides") OR EXCLUDE (EXACTKEYWORD , "Insecticide") OR EXCLUDE (EXACTKEYWORD , "Bovine Mastitis") OR EXCLUDE (EXACTKEYWORD , "Unclassified Drug"))

8.2 Findings in humans

Table X.92: Overview toxicokinetics, metabolism and biomonitoring studies in humans

Reference	Cited in	Findings
Song et al. 2020	(Zhang and Lu, 2022)	- Median values for imidacloprid in urine samples of Chinese general population were found to be 0.21 ng/mL.urine
Li and Kannan 2020c	(Zhang and Lu, 2022)	- In 118 urine samples of Greek residents (general population) the concentration of imidacloprid was 0.144 ng/mL
(Harada et al., 2016)		- A single micro dose of a mixture of deuterium-labeled neonicotinoids (5 µg each of acetamiprid-d6, clothianidin-d3, dinotefuran-d3, and imidacloprid-d4) was orally ingested by each of nine healthy adults - 24 h pooled urine samples were collected for 4 consecutive days after dosing - Excretion kinetics were modelled using one- and two-compartment models, then validated in a non-deuterium-labeled neonicotinoid micro dose study involving 12 healthy adults ingesting a single oral dose of a mixture of 2 µg each with 24h pooled urine samples collected on the day before and after the micro dose exposure with a spot urine sample being collected every 24 h until 168 h after ingestion for each participant - 12.7% of the imidacloprid dose was excreted unchanged within 96h - A large proportion of the ingested imidacloprid would have been converted into unidentified metabolites
(Wrobel et al., 2022)		- Human metabolism and metabolite excretion in urine of acetamiprid (ACE), clothianidin (CLO), flupyradifurone (FLUP), imidacloprid (IMI), sulfoxaflor (SULF), THD (THIAC) and THIAM (THIAM) after single oral dosages at the currently ADI levels of the EFSA - Conducted for each of the seven NEOs in a single volunteer (male, Caucasian, 50 years, 83 kg body weight, non-smoker) - Doses were applied orally - Post-dose urine samples were collected consecutively and completely up to 48 h - The parent compound and three putative metabolites, hydroxylated IMI (4-/5-OH-IMI), IMI olefin and 6-CNA-glycine could be detected in post-dose urine - Observed prolonged excretion of unchanged IMI up to 48 h is in line with the results of Harada et al. (2016) who described the excretion of IMI in urine up to 96 h after oral ingestion of deuterated IMI - A dihydroxylated metabolite of IMI such as 4,5-OHIMI could not be detected
(Wrobel et al., 2023)		- Quantitative investigation of the human metabolism and urinary excretion kinetics of seven globally important NEOs, i.e., acetamiprid (ACE), clothianidin (CLO), flupyradifurone (FLUP), imidacloprid (IMI), sulfoxaflor (SULF), THD (THIAC), and THIAM (THIAM)

Reference	Cited in	Findings
		- Urine sample from (Wrobel et al., 2022) were used, two male volunteers (37 years, 98 kg; 48 years, 96 kg) were additionally administered individual single oral doses of selected NEOs (ACE, CLO, FLUP, and IMI) - For IMI, 4-/5-OH-IMI and IMI-olefin the cmax values were 560 ± 47, 727 ± 141 and 557 ±197 µg/g creatinine, respectively, the non-specific metabolite 6-CN-gly had a cmax value of 11 ±2.0 µg/g creatinine - Tmax for IMI, 4-/5-OH-IMI and IMI-olefin was 2.0 ± 0.4, 7.9 ± 2.7 and 8.4 ± 2.5 h, respectively, values for t1/2 were 12.2 ± 2.0, 22.7 ± 9.2 and 12.4 ± 1.5 h, respectively - For IMI, a Fue of 6.6 ± 0.2 % was calculated after 24 h and 8.3 ± 0.6 % after 48 h - For 4-/5-OH-IMI, Fue was 22 ± 4% and 31 ± 4% after 24 and 48 h, respectively, For IMI-olefin, 18 ± 7% and 27 ± 8% after 24 and 48 h, respectively - According to the authors, the recommended biomarker for assessing the exposure to IMI is IMI-olefin which reached a urinary excretion fraction of 27%, but 4-/5-OH-IMI were also considered good and robust biomarkers for exposure and risk assessment
(Wrobel et al., 2024)		- Case study on male volunteers after applying a ready-to-use IMI-containing spot-on solution (2.5 mL containing 1 g/L of IMI) was applied without wearing gloves at three spots to a dog's back directly on the skin by manually splitting the hair according to the manufacturer's instructions - Urine samples were collected directly before the application (*t*0) and consecutively and completely during the following 48 h - Concentrations of IMI, OH-IMI, and IMI-olefin were below the LOQ at t0. OH-IMI was the first metabolite to emerge with concentrations above the LOQ in two samples at 13 and 15 h after dog treatment (or 4.5 and 6.5 h after petting and cuddling with the dog). All three analytes were then quantifiable in all urine samples 20 h post-application (or 11.5 h after the first post-application contact with the dog) - Creatinine-corrected urinary concentrations rather constantly increased to reach their maximum 38 h after the application, with corresponding *c*max of 3.1, 14.9, and 8.0 µg/g creatinine for IMI, OH-IMI, and IMI-olefin, respectively, slowly decreasing thereafter but remaining above LOQ 56 h after application - Total excretion of IMI, OH-IMI, and IMI-olefin (until the last sampling point 56 h post-application) was 73.5, 439.6, and 212.0 µg, respectively, corresponding to an oral dose equivalent IMI intake of 10.6 µg/kg body weight based on the summed three urinary biomarkers
(Chen et al., 2023)		- 201 participants and quantified eight commercialized NEOs in bile – IMI 0.27 – 13.8 µg/L

Reference	Cited in	Findings
(Gu et al., 2023)		- Study investigated the association between the urinary neonicotinoid and the relative mtDNAcn (RmtDNAcn) of oral epithelial cells collected from 35 postgraduates (25 male and 10 female) in a cross-sectional study with repeated measurements over 6 weeks - DF of IMI was 54.3–74.3%; The mean concentrations were detected, ranging from 0.28 – 0.50 µg/L in urine
(Chen et al., 2020)		- Nationwide survey from 23 Chinese provinces with 97 pooled breast mild samples from 3570 lactating women from 2017-2019 - N-desmethyl-acetamiprid was the most predominant compound, accounting for 61.2% of the total amount of neonicotinoids, followed by imidacloprid with only 15.6% - No statistically significant association in breast milk with corresponding dietary exposure - The detection frequency in the 97 pooled samples was 99% for IMI
(Gao et al., 2022)		- Urine samples from 25 volunteers - Imidacloprid median: 7 pg/mL
(Gao et al., 2023)		- The first morning voids urine (FMVU), spot urine (SU), and 24 h urine (24hU) samples from 8 healthy adults during 7 consecutive days - Dinotefuran (DIN) and olefin-imidacloprid (of-IMI) were excreted in the highest concentration; Of-IMI median concentration in the range 0.11-0.18 ng/mL
(Henríquez-Hernández et al., 2022)		- Cohort of 403 subjects from Cape Verde - Imidacloprid was detected in 14.4% of the population; mean blood concentration of imidacloprid was 0.18 ng/mL
(F Li et al., 2022)		- To understand the reliability of a single measurement of urinary neonicotinoid biomarkers in representing a true longer-term average exposure, the temporal variability of 14 neonicotinoids and/or their metabolites over one year in 114 Chinese young adults were evaluated - Among others, 5-OH-IMI required a minimum of 2–4 spot urine samples over one year to obtain a reliable exposure evaluation. - The authors recommended at least 2–4 spot urine samples to be used to assess 1-year neonicotinoid exposure and to consider seasonal variations when scheduling urine sample collection - 757 samples were available, IMI was detectable in 44% of them, its metabolite in 92%, with adjusted mean values of 0.17 and 0.60 µg/g creatinine

Reference	Cited in	Findings
(López-Gálvez et al., 2020)		- 20 male migrant grape workers in Sonora Mexico sampled five days after imidacloprid was injected in the irrigation system during winter and summer seasons - The majority of participants (75%) had detectable urinary concentrations of imidacloprid (median: 0.11 µg/g creatinine, range: 0.05–3.90 µg/g of creatinine), and nearly all (95%) had detectable urinary concentrations of 5-hydroxy-Imidacloprid (5-OH-IMI), a metabolite of imidacloprid (median: 1.28 µg/g creatinine, range: 0.20–27.89 µg/g creatinine)
(AJ Li et al., 2020)		- Urinary levels of creatinine-adjusted IMI 0.065-0.071 µg/g
(Mahai et al., 2022)		- Nine parent NEOs and ten main metabolites in 1224 urine samples donated by 408 pregnant women at three trimesters were measured - The unadjusted vs. specific gravity (SG) adjusted median concentrations and detection frequencies (DFs) of 5-hydroxy-imidacloprid (0.54 vs. 0.56 ng/mL; DF: 98.5%), imidacloprid-olefin (0.41 vs. 0.44 ng/mL; DF 99.3%), and desnitro-imidacloprid (0.12 vs. 0.12 ng/mL; DF 90.4%) was higher than corresponding parent NEOs, imidacloprid (IMI, 0.04 vs. 0.04 ng/mL; 69.9%) - A decreased trend was observed in urinary NEOs and their metabolites throughout the three trimesters, maternal age, educational level, and household income were related to the concentrations
(Manfo et al., 2024)		- 188 men, including 125 farmers and 63 nonfarmers from two agrarian regions of Cameroon, the South-West region and Littoral region - Neonicotinoid compounds were detected in all study participants, and residues of all the screened pesticides were detected among participants. N-dm-Acetamiprid and imidacloprid were the most prevalent among the subjects (100.0% and 93.1%, respectively). - The median values of imidacloprid and total urinary neonicotinoid concentrations were elevated among farmers (0.258 vs. 0.126 µg/L and 0.829 vs. 0.312 µg/L, respectively).
(Nimako et al., 2021a)		- A total of 75 human urine samples from healthy non-farming volunteers (19 males, 56 females) from Ghana aged 18-80 were examined for several neonicotinoids - Imidacloprid detected in 70.7% of the samples with a median concentration of 0.15 µg/L, and maximum concentration of 211.62 µg/L

Reference	Cited in	Findings
(Song et al., 2020)		- 275 urine samples collected from residents aged 1-91 years in 10 cities in China - 5-hydroxy-imidacloprid was detected in 88% of the samples with a median urinary concentration of 1.02 ng/mL - olefin-imidacloprid DF 63% a median urinary concentration of 0.48 ng/mL - The ratio of m-NEO to its corresponding parent NEO (m-NEO/NEO) was 37.7, indicating that the urinary m-NEO level was significantly higher than that of the corresponding parent compound
(Suwannarin et al., 2020)		- Urinary concentrations of 100 reproductive-age farmworkers from two intensive farming areas in Chiang Mai Province, Thailand, including 50 each - NEO exposure was determined by measuring the urinary concentrations of NEO compounds and their metabolites (NEO/m) - IMI had a detection frequency of 94% with the highest GM concentration, 8.7 µg/g-creatinine - However, the max concentration measured was below the geometric mean and below the confidence interval, so the values are not trustworthy
(Thompson et al., 2023)		- Private-well tap water and human urine samples of 50 participants in a region of intense agriculture overlying vulnerable aquifers in eastern Iowa - Estimated daily intake via water of NEOs was 0.004, 0.001, 0.001, 0.001 and 0.001 ng/kg bw/d for imidacloprid in infants, toddlers, children, teenagers and adults, respectively, the maximum EDI values were 2.635, 0.908, 0.849, 0.469 and 0.586 ng/kg bw/d - In the urine samples, imidacloprid had a detection rate of 53% with a maximum concentration of 7.4 µg/g creatinine - According to the authors, the study results indicate that while the consumption of private well tap water provides a human exposure pathway, the companion urine results provide evidence that diet and/or other exposure pathways (e.g., occupational, house dust) may contribute to exposure more than water contamination but further biomonitoring research would be needed
(A. Wang et al., 2020)		- 408 urine samples of 129 healthy adults from Wuhan, China during autumn and Winter 2018 - Expected, the amounts of the metabolites were higher than that of the parent compound - DF of DN-IMI was 100% and IMI-olefin accounted for 93% of the urinary - The concentration ranges were below the detection limit until 0.86 ng/mL for IMI and until 0.03–7.48 ng/L for DN-IMI, and IMI-olefin 10.3 ng/mL - According to the authors, DM-ACE can be used as biomarker for human biomonitoring of IMI

Reference	Cited in	Findings
(H. Wang et al., 2020)		- 309 school children aged 7–11 years were selected from a dynamic cohort of children established in Shanghai, East China in 2019 - Eight neonicotinoids and four metabolites were determined in first morning urine - IMI was detected in 3.5 % of the probes - Some demographic and socioeconomic factors, physical exercise, and BMI were found to be predictors for the exposure to neonicotinoids - Tap water and fresh vegetables were found to be potential exposure sources
(Y. Wang et al., 2024)		- 476 Tibetan pregnant women from Lhasa of Tibet, China in 2021 and measured eight neonicotinoids and four metabolites in urine - IMI detected in 10.5% samples with maximum concentration 2.53 ng/mL, and of 5-OH-imidacloprid in 0.2% with maximum concentration 2.04 ng/mL
(A. Wang et al., 2024)		- 1,094 Chinese pregnant women were recruited and a total of 3,282 urine samples were collected at their three trimesters to measure eight metabolites of organophosphates, three metabolites of pyrethroids, nine typical neonicotinoids/their metabolites - IMI, detection frequency: 72.9%, desnitro-imidacloprid (DN-IMI) 70.0% - The concentrations for mNEOs ranged 0.03–1.09 ng/mL.
(Wrobel et al., 2022)		- Human metabolism and metabolite excretion in urine of acetamiprid (ACE), clothianidin (CLO), flupyradifurone (FLUP), imidacloprid (IMI), sulfoxaflor (SULF), THD (THIAC) and THIAM (THIAM) after single oral dosages at the currently ADI levels of the EFSA - Conducted for each of the seven NEOs in a single volunteer (male, Caucasian, 50 years, 83 kg body weight, non-smoker) - Doses were applied orally - Post-dose urine samples were collected consecutively and completely up to 48 h - The parent compound IMI and three putative metabolites, hydroxylated IMI, IMI olefin and 6-CNA-glycine - Unchanged IMI and both hydroxylated IMI, IMI olefin were detected up to 48 h post-dos e in urine - the excretion profile of 6-CAN-glycine was inconclusive compared to the other metabolites - Results are in line with (Harada et al., 2016)
(Wrobel et al., 2024)		- Case study on male volunteers after applying an IMI-containing spray agent (2.5mL containing 1g/L of IMI) on was applied at three spots to the dog's back directly on the skin by manually splitting the hair according to the manufacturer's instructions without dermal protection

Reference	Cited in	Findings
		- Urine samples were collected directly before the application of IMI (t0) and consecutively and completely during the following 56h - IMI, OH-IMI, and IMI-olefin were below the LOQ at t0. All three analytes were then quantifiable in all urine samples 20h post-application. - The total excretion of IMI, OH-IMI, and IMI-olefin (until the last sampling point 56h post-application) was 73.5, 439.6, and 212.0µg, respectively, corresponding to an oral dose equivalent IMI intake of 10.6µg/kg body weight based on the summed three urinary biomarkers.
(Wrobel et al., 2023)		- Quantitative investigation of the human metabolism and urinary excretion kinetics of seven globally important NEOs, i.e., acetamiprid (ACE), clothianidin (CLO), flupyradifurone (FLUP), imidacloprid (IMI), sulfoxaflor (SULF), THD (THIAC), and THIAM (THIAM) - Urine sample from (Wrobel et al., 2022) were used, two male volunteers (37 years, 98 kg; 48 years, 96 kg) were additionally administered individual single oral doses of selected NEOs (ACE, CLO, FLUP, and IMI) - For IMI the cmax was 560 ± 47 µg/g creatinine, respectively, three non-specific metabolites (4-/5-OH-IMI, IMI-olefin and 6-CNA-gly) had cmax values of 727 ± 141, 557±197 and 11 ± 2.0 µg/g, respectively - Tmax for IMI was 2.0 ± 0.4 h, values for t1/2 were 12.2 ± 2.0 h; Tmax for 4-/5-OH-IMI was 7.9 ± 2.7 h, values for t1/2 were 22.7 ± 9.2 h; Tmax for IMI-olefin was 8.4 ± 2.5 h, values for t1/2 were 12.4 ± 1.5 h - For IMI, a Fue of 6.6 ± 0.2 % was calculated after 24 h and 8.3 ± 0.6 % after 48 h - For 4-/5-OH-IMI Fue were 22 ± 4 % and 31 ± 4 % after 24 and 48 h, respectively - For IMI-olefin Fue were 18 ± 7% and 27 ± 8% after 24 and 48 h, respectively - Based on the Fue for IMI-olefin a median daily intake of 1.43 µg/kg bw/d was calculated for IMI - Using the parent compound or the sum of 4- and 5-OH-IMI instead, similar maximum DIs were estimated (1.3 or 1.81 µg/(kg bw·d))
(Xu et al., 2021)		- Six NEOs and three characteristic metabolites were detected in 196 paired urine and blood samples collected from young adults from China - Olefin-imidacloprid (Of-IMI) and 1-methyl-3-(tetrahydro-3-furylmethyl) urea (UF) were the most abundant target compounds in the urine (32.4%) and blood (26.4%) samples, respectively - The median EDI values (ng/kg bw/day) of ΣNEOs (sum of NEOs and their metabolites) and IMIeq in females (194.9 and 458.2) and in males (157.1 and 439.7)

Reference	Cited in	Findings
(Ying et al., 2023)		- New method to detect NEOs in the breast milk - 32 samples obtained from women 1 month postpartum, THIAM and imidacloprid were the most frequently detected NEOs - Detection rate for imidacloprid was 56.25% with average concentration 29.07 ng/mL and maximum concentration 82.07 ng/mL
(Zahid et al., 2024)		- The human urinary levels of neonicotinoids in samples collected during 2022–2023 in the population living in areas close to this now-closed bioenergy plant near Mead, Nebraska - 5-hydroxy-imidacloprid, the transformed imidacloprid product, was detected in 27% of the samples, ranging from 1.2 to 42 ng/mL
(Henglin Zhang et al., 2022)		- Parent NEOs (p-NEOs) and their metabolites (m-NEOs) were determined in 95 paired maternal (MS) and cord serum (CS) samples collected in southern China - Imidacloprid was the predominant p-NEO in both CS and MS samples, found at median concentrations of 1.84 and 0.79 ng/mL - The median transplacental transfer efficiencies (TTEF) of p-NEOs and m-NEOs were high, ranging from 0.81 (THIAM, THM) to 1.61 (olefin-imidacloprid, of-IMI), indicating efficient placental transfer of these insecticides.
(Zhang et al., 2023)		- Concentrations of six parent NEOs (p-NEOs) and N-desmethyl-acetamiprid (N-dm-ACE) were measured in urine and whole blood samples from infants, in addition to breast milk, infant formula, and tap water collected in South China - Imidacloprid (IMI) was abundant in breast milk (median: 0.27 ng/mL), infant formula (0.22 ng/mL)
(Q. Zhang et al., 2023a)		- 115 adult whole blood and plasma specimens from individuals living in Anji, Zhejiang, China, participated in this study from April to May 2018 for analysis of eight neonics to analyse partitioning and distribution in blood samples - Imidacloprid had the highest detection rate with 26.1% in the plasma but no information on its metabolite in the study
(Zhang et al., 2021)		- 40 paired samples (i.e., 160 urine and 40 indoor dust) were collected from university students and dormitories from Guangzhou City of China to measure the concentrations of six NEOs and their three metabolites. - 5-Hydroxy-imidacloprid was predominant in urine (56%) - The highest median urinary concentration was found for 5-OH-IMI (2.08 ng/mL; SG-adjusted: 2.38 ng/mL). Moreover, the median urinary concentration of m-NEOs (i.e., 5-OH-IMI, ACE-dm, and UF) was significantly higher than their corresponding p-NEOs (IMI, ACE, and DIN)
(Q. Zhang et al., 2023b)		- The breast milk samples were collected from Hangzhou Medical College from mothers within 2 months of delivery (137 breast milk samples) - Imidacloprid detection rate was 62.0 % with the concentrations ranging from 8 (LOD) – 335 ng/L

Reference	Cited in	Findings
(Zhao et al., 2022)		- Children aged from 8 to 11 years old were recruited from six administrative divisions in Shenzhen, China. - The median concentration of IMI was 0.13 µg/L - DF of IMI was 95.7%
		- Measuring the concentration of NEOs in urine of 386 adolescents in Chongqing of Southwest China - The geometric mean of IMI was 3.80 µg/g creatinine

Table X.93: Overview epidemiological studies evaluating neonicotinoid exposures and effects

Reference	Cited in	Findings
Von Ehrenstein et al. 2019	(Ongono et al., 2020b)	• Case-control studies investigated the effects of early exposure to imidacloprid on the ASD (autism spectrum disorder) risk • Conducted in California (US) 2961 ASD cases [including 445 with intellectual disability (ID)]. Exposure assessment method: GIS with data from local agricultural application records (CA-PUR reports), combined with the location of specific crops. Windows of exposure: prenatal period, and the first year of life. • Any significant association between early exposure to imidacloprid used on agricultural parcels nearby maternal residence and the ASD risk, with an estimated OR close to 1 • The evaluation of the study by authors of the review: the level of evidence in the body of evidence was considered "inadequate" (Von Ehrenstein et al., 2019; Keil et al., 2014) meaning an insufficient number of evidence to draw a conclusion in favour or against this association.
Keil et al. 2014	(Cimino et al., 2017; 2020 NTP, 2020; Ongono et al., 2020b)	• Case control study was interested in maternal use of imidacloprid in the domestic context during the prenatal period and the three first years of life • Conducted in California (US) 407 ASD children from the CHARGE study and 262 controls; data collected through maternal phone interview. Windows of exposure: from 3 months before conception through 3 years of age. • Non-statistically significant increase of 30% in the risk of ASD among the children of women using imidacloprid in the domestic context during pregnancy (OR =1.3, 95%CI: 0.78, 2.2) • The evaluation of the study by authors of the review: the level of evidence in the body of evidence was considered "inadequate" (Von Ehrenstein et al., 2019; Keil et al., 2014) meaning an insufficient number of evidence to draw a conclusion in favour or against this association.
Carmichael et al. 2014	(Cimino et al., 2017; 2020 NTP, 2020)	• Case-control (California/Infants or foetuses with congenital heart defects from the California Birth Defects Monitoring Program) [101 cases, 785 controls] • Exposure estimated categorically (none/any) from state pesticide use records between 1997–2006 and proximity of residence to use (in utero) • AdjORs for imidacloprid and neonicotinoid pesticide group exposures and congenital heart defects
Khan et al. 2010	(2020 NTP, 2020)	• Cross-sectional (Pakistan/adult pesticide industrial workers) [238 males; 184 exposed, 54 unexposed] • Exposure groups estimated based on industrial size and production of pesticides as opposed to specific chemical exposure, but imidacloprid was measured in plasma of 184 industrial workers (adult) • Increased level of liver enzymes, oxidative stress markers, inflammatory markers

Reference	Cited in	Findings
		• Decreased level of plasma cholinesterase levels, total antioxidants • No change in plasma bilirubin, albumin, total proteins, urea, creatinine • Clinical symptoms including headache followed by dizziness, vomiting, shortness of breath, skin rash were commonly reported (in about 2–20% depending on clinical symptom and size of industry)
Marfo et al. 2015	(Cimino et al., 2017; EFSA Panel on Plant Protection Products and their Residues (PPR) et al., 2022), (2020 NTP, 2020)	• Multiple insecticides including imidacloprid measured in spot urine samples collected from patients (4-87 years old) attending a clinical study in Japan over a period of three years (exposure timing unknown) • Patients lived close to agricultural fields • Categorised in three groups: typical symptomatic group (TSG, n=19), atypical symptomatic group (ASG, n=16), non-symptomatic group (NSG, n=50) • Exposure to multiple insecticides including acetamiprid, THD, imidacloprid, nitenpyram, clothianidin, THIAM measured in spot urine samples • (exposure timing unknown). • Nicotinic symptoms in subjects with increased levels of THIAM and dme-ACE, a metabolite of acetamiprid
Shaw et al. 2014	(2020 NTP, 2020)	• Case-control (California/Infants or foetuses with gastroschisis from the California Birth Defects Monitoring Program) [156 cases, 785 controls] • Exposure estimated categorically (none/any) from state pesticide use records between 1997–2006 and proximity of residence to use (in utero) • Evaluated effect - Birth defect (gastroschisis); increased AdjORs for imidacloprid and neonicotinoid pesticide group exposures and gastroschisis
Yang et al. 2014	(Cimino et al., 2017; 2020 NTP, 2020)	• Case-control (California/Child participants of the California Birth Defects Monitoring Program) [590 cases, 785 controls] • Exposure - Neonicotinoid pesticide group including imidacloprid Exposure estimated categorically (none/any) from state pesticide use records between 1997–2006 and proximity of residence to use (in utero) • Outcome - Birth defects (anencephaly, spina bifida, cleft lip with or without cleft palate, or cleft palate only) • Increased AdjORs for anencephaly, cleft lip with or without cleft palate, cleft palate only no odds ratio calculated for spina bifida
(Cimino et al., 2017)	(X. Zhang et al., 2023)	• Summary of eight pieces of literature that investigated the impact of neonicotinoid pesticide toxicity on the human health • Four of these examined acute exposure (no adverse effects were observed in one item, and adverse effects were observed in three items), and the other four reported an association between chronic neonatal exposure and adverse development

Reference	Cited in	Findings
		or neurological prognosis (both show a good correlation between exposure to neonicotinoid insecticides and adverse health effects in humans).
(Chen et al., 2021)		• Serum concentrations of neonicotinoids, and their associations with lipid molecules • 120 human serum samples were collected from the area of Wuxi city, Eastern China • Imidacloprid exhibited relatively high detection frequency (28.3%); Median concentrations of imidacloprid-equivalent total neonicotinoids (IMIeq) and ∑7NEOS were 46.6 pg/mL and 26 pg/mL, respectively. Five out of nine lipid molecules exhibited higher levels but the study did not express the link with IMI.
(Godbole et al., 2022)		• The association between concentrations of parent neonicotinoids (imidacloprid, acetamiprid, clothianidin) and neonicotinoid metabolites (5-hydroxy-imidacloprid, N-desmethyl-acetamiprid) with adiposity measures among 9544 US adults (NHANES 2015-2016) • Detectable levels of 5-hydroxy-imidacloprid were associated with greater rates of being overweight/obese (IRR = 1.11, CI [1.04, 1.18)) and increased LMI (β = 0.67 kg/m2, CI [0.04, 1.29]).
(Hyland et al., 2021)		• Study investigating associations with maternal- and youth-reported behavioural and emotional problems from the Behavior Assessment System for Children, 2nd edition (BASC-2) at ages 16 and 18 years (n = 593) among participants in the Center for the Health Assessment for Mothers and Children of Salinas (CHAMACOS) birth cohort • It was observed mostly null associations between pesticide applications and neurobehavioral outcomes; some protective associations with imidacloprid during the prenatal period, particularly in sex-specific analyses, were observed.
(G Mahai et al., 2023)		• Study focused on the associations of urinary concentrations of NEOs in early pregnancy with gestational diabetes mellitus (GDM) and the mediation roles of oxidative DNA damage, RNA damage, and lipid peroxidation in the associations. • Nested case-control study, 519 GDM cases and 519 controls were matched on the infant's sex and maternal age. • The odds of GDM significantly increased by 15 %, 18 %, 26 %, 42 %, 49 %, and 13 % in each unit increment of ln-transformed concentrations of urinary imidacloprid (IMI), imidacloprid-olefin (IMI-olefin), desnitro-imidacloprid (DN-IMI), THIAM (THM), clothianidin, and desmethyl-clothianidin, respectively
(Mendy and Pinney, 2022)		• Study investigating the association of urinary neonicotinoids with serum testosterone in 2014 male and female participants to the National Health and Nutrition Examination Survey conducted between 2015 and 2016 aged 6 or older. • Serum total testosterone was 20.81% lower with 10-fold increase in urinary 5-hydroxy-imidacloprid among males. • In females, serum total testosterone was 21.32% lower with 10-fold increase in urinary 5-hydroxyimidacloprid and 15.42% lower with urinary detection of 5-hydroxy-imidacloprid. FAI (free androgen index) was likewise reduced with higher urinary levels of 5-hydroxy-imidacloprid

Reference	Cited in	Findings
(Mu et al., 2024)		• Prospective cohort study of 500 mother-newborn pairs from the Guangxi Zhuang Birth Cohort focused on the effects of prenatal exposure to NEOs and the interactions between NEOs and sampling season on newborn telomere length (TL) • Exposure of pregnant women to IMI changed the newborn TL, and sampling season may modify this change
(Pan et al., 2022)		• 296 mother-infant pairs recruited from Laizhou Wan Birth Cohort in 2010 – 2013. Two NEOs (IMI and ACE), three metabolites (6-CN, ND-ACE, and 2CTCA), and 8-OHdG were measured in maternal urine collected before delivery • Highest detection rate was observed for ACE (100.0%), followed by IMI (98.3%) and 6-CN (98.0%) • A decrease in newborns' head circumference was observed with a 10-fold increase in IMI • An increase in newborns' PI (ponderal index) was observed with a 10-fold increase in IMI • Maternal 8-OHdG demonstrated 38.5–65.5% mediating effects in the negative association of IMI, ACE, 2-CTCA with head circumference
(Qu et al., 2024)		• NEO exposure in pregnant women during early-mid pregnancy and to assess the associations between NEOs and congenital heart diseases (CHDs) • Nested case-control study was conducted within an ongoing prospective birth cohort study and enrolled 141 CHD singletons and their 282 individually matched controls • Not found a statistically significant association between total NEOs and overall CHDs. However, there was a trend towards a higher risk of septal defects with greater serum NEOs (ORs ranged from 1.80 to 2.36), especially nitro-containing NEOs represented by IMI. Pregnant women with lower education had elevated serum total NEOs compared to women with higher education (OR = 48.39, 95% CI: 23.48–99.72). • No associations found for IMI
(Sun et al., 2023)		• Study investigating the relationship between NEOs exposure and dyslipidemia 500 individuals (236 and 264 with and without dyslipidemia, respectively) randomly selected from the baseline of the Yinchuan community-dwelling elderly cohort (Ningxia, China). • NEOs mixtures were associated with increased dyslipidemia when the chemical mixtures were ≥ 25th percentile compared to their medians, and desnitro-imidacloprid and imidacloprid-olefin were the major contributors to the combined effect
(Suwannarin et al., 2021)		• Cross-sectional study looking at the exposure to organophosphate and neonicotinoid insecticides and its association with steroid hormones • 143 male farmworkers aged 18–40 years in Fang district, Chiang Mai province, northern Thailand; the concentration of imidacloprid (IMI) was GM: 17.4 ng/mL

Reference	Cited in	Findings
		• IMI level was positively associated with testosterone, dehydrocorticosterone (DHC) and dehydroepiandrosterone (DHEA) levels. Imidacloprid-olefin and DHEA levels were positively associated.
(Taira et al., 2021)		• The field-based descriptive study in the North Central Dry-zone of Sri Lanka to elucidate the relationship between renal tubular dysfunctions and urinary neonicotinoids concentrations • Urine samples were collected from15 CKD (chronic kidney disease) patients, 15 family members, and 62 neighbours in 2015 • No association identified for IMI
(Vuong et al., 2022)		• Associations between neonicotinoids with insulin and glucose homeostasis parameters among 1381 non-diabetic adults in the National Health and Nutrition Examination Survey (2015–2016). • Detectable imidacloprid and 5-hydroxy-imidacloprid were associated with lower fasting plasma insulin levels. Individuals with detectable 5-hydroxy-imidacloprid had lower odds of insulin resistance (OR=0.3, CI 0.2, 0.7). Evidence of sexually dimorphic associations was observed between N-desmethyl-acetamiprid with glucose and 5-hydroxy-imidacloprid with HbA1c , with patterns suggesting positive associations in males and negative associations in females. • Associations between 5-hydroxy-imidacloprid and insulin were modified by BMI. Additionally it was observed age modified associations between 5-hydyroxy-imidacloprid and glucose.
(Wang et al., 2022)		• The potential effects of NEOS exposure on male semen quality was investigated in cohort of men (n = 191) who visited a fertility clinic in Shijiazhuang, North China from 2018 to 2019. • The IMI-olefin concentration was associated with decreased progressive motility.
(Wu et al., 2024)		• Cross-sectional study investigating the exposure to neonicotinoids and its association with obesity among 524 Chinese adolescents (300 boys, 224 girls). • The concentrations of imidacloprid (OR = 0.35, CI: 0.14, 0.88) were associated with lower odds of being general obesity.
(Yang et al., 2024)		• Study investigating the exposure to neonicotinoids and its association with obesity among 442 children in Shanghai, East China • No association identified for IMI
(Yang and Liang, 2022)		• Cross-sectional study investigating associations between neonicotinoids metabolites and hematologic parameters among US adults in 1397 NHANES 2015–2016 • Detectability of 5-hydroxy-imidacloprid was inversely correlated with basophil percentage. Imidacloprid-equivalent total neonicotinoids (IMIeq) were negatively related to red blood cells, haemoglobin, and haematocrit (only in males).

Reference	Cited in	Findings
(Yue et al., 2022)		• A cross-sectional study investigating urinary neonicotinoid concentrations and pubertal development in 774 Chinese adolescents (439 boys, 335 girls). • No association identified for IMI
(Hua Zhang et al., 2022b)		• Study investigating the associations between the exposure to neonicotinoids and osteoporosis (OP) in 200 elderly people (age: ≥ 50 years) from South China • No association identified for IMI
(Hua Zhang et al., 2022a)		• Study investigating the associations between the exposure to neonicotinoids and liver cancer in 374 participants from one hospital in Guangzhou, Guangdong province of China; 274 were liver cancer patients, who were newly diagnosed prior to treatment; while the remaining were healthy. • No association identified for IMI
(Zhao et al., 2023)		• Cross-sectional study investigating the relationship between NEOs and their metabolites in urine with serum lipid profiles in adults using data from the National Health and Nutrition Examination Survey (NHANES) 2015–2016. The study included 1192 participants aged over 20 years. • No significant associations were observed between other NEOs than acetamiprid and lipid profiles in the study
Elfman 2009	(Cimino et al., 2017)	• 19 conifer seedling planters: 17 men, 2 women in Sweden • No clear acute adverse effects reported after 1 week of exposure to IMI-treated seedlings
Forrester 2014	(Cimino et al., 2017)	• 1,142 exposure cases reported to a TX poison control network from 2000 to 2012 in USA • Of the 1,142, 77% were identified as IMI alone or in combination with other neonics. • Thirty-two neonic exposures (2.9%) resulted in "serious medical outcomes" including ocular irritation/pain, dermal irritation/pain, nausea, vomiting, oral irritation, redeye, erythema, rash, numbness, and dizziness. Chest pain (2 exposures; 0.2%),hypertension (0.2%), and tachycardia (0.2%) were the most frequently reported serious cardiovascular effects. No deaths reported

Reference	Cited in	Findings
Mohamed 2009	(Cimino et al., 2017; EFSA Panel on Plant Protection Products and their Residues (PPR), 2013)	• 68 hospital patients: 61 ingestion, 7 dermal exposures in Sri Lanka • Of the 56 patients with acute IMI poisoning (versus mixtures), only 2 developed severe symptoms. • The majority had mild symptoms including nausea, vomiting, headache, dizziness, abdominal pain, and diarrhoea. • IMI exposure confirmed in 28 cases, with a median plasma concentration of 10.58 ng/L (IQR: 3.84–15.58 ng/L; range: 0.02–51.25 ng/L) on admission. Concentrations for 7 patients remained elevated for 10–15 hr post-ingestion, suggesting absorption and/or elimination may be saturable or prolonged at high doses. No deaths reported.
Phua 2009	(Cimino et al., 2017)	• 70 exposure cases reported to the Taiwan National Poison Center in China • Of the 57 cases of ingested neonics, the majority were of IMI (n = 53), followed by ACE (n = 2) and CLO (n = 2). The 10 most severe cases were from IMI alone. Two deaths reported (mortality rate 2.9%).
(Bhatta et al., 2023)		• Poisoning in a 25-year-old previously healthy indigenous Tamang female without a classic toxidrome requiring ventilatory support, complicated by a prolonged neuropsychiatric sequela. • After extubating, she remained hypoxemic and required oxygen supplementation for 7 days. On psychiatric evaluation during the ICU stay, the patient was delirious. discharged on the tenth day of admission without any residual effects and was well oriented.
(Chadalavada and Baddam, 2023)		• A 14-year-old boy presented to the hospital after deliberate ingestion of 50 mL of imidacloprid mixed with alcohol. In an unconscious state approximately 30 minutes after the alleged intake. The patient underwent gastric lavage. Despite high-flow oxygen, saturation levels remained between 70% and 80%. The patient was intubated and received antibiotics for suspected aspiration following alcohol intoxication.
(James et al., 2023)		• Case report of a patient (an 18-year-old boy) who presented to the emergency department with features suggestive of neuromuscular junction dysfunction, following exposure to imidacloprid as the part-time worker at the family's vegetable garden. He was with double vision and drooping of both eyelids for the past 18 hours. He recovered over two days and was discharged.
(Kasiotis et al., 2021)		• Case report of 86-year-old female admitted to the hospital after reportedly receiving organophosphates (chlorpyrifos) and an additional formulation with potential active ingredient myclobutanil (triazole fungicide). She had an altered level of consciousness and was gasping for air. She was intubated at the emergency department. Soon after the intubation, the patient went into cardiac arrest and was resuscitated immediately. At day 15, she was discharged upon further improvement. • Concentration of imidacloprid measured in gastric fluid (no. 1) 23.2 ± 1.7 (ng/mL), (no. 2) 25.7 ± 2.1; tR(min) 10.62

Reference	Cited in	Findings
(Naveen et al., 2022)		• Case reports of 4 cases with self-poisoning: 18-year-old woman self-poisoned with an unknown quantity of IMI (17.8%) insecticide. She developed vomiting and abdomen discomfort after ingestion. Laboratory investigations revealed leukocytosis, decrease in haemoglobin level and red blood cell count, and impairment of both renal and liver function tests. She died after 37 days of poisoning; 26-year-old man of self-ingestion of approximately 50 mL of VICTOR (IMI 17.8%) insecticide poison - Neurological examination revealed muscular weakness. He was discharged after 72 hours of hospital stay; 52-year-old man succumbed on the fourth day of poisoning due to the combined effect of IMI poisoning and COVID-19 pneumonia; 73-year-old male farmer with symptoms of nausea and abdominal discomfort. He was after the 48 hours discharged from hospital.
(Shiri-Ghaleh et al., 2021)		• Case report of clinical and postmortem findings of a fatal imidacloprid poisoning in a 33-year-old female. The toxicological analysis showed that imidacloprid concentrations were 267.8 µg/mL in femoral blood, 144.5 µg/mL in stomach content, and 292.3 µg/mL in bile (no information presented about the ingested volume).
(C. Sriapha et al., 2020)		• A retrospective poison center case series identified 128 cases of imidacloprid ingestion from 2010–2016, of which four developed liver injury. Three patients ingested soluble liquid concentrates and one ingested water-dispersible granules of imidacloprid. The estimated doses of ingestion ranged from 2–35 g. One patient developed cholestatic liver injury, two developed hepatocellular liver injury, and the remaining patient, who ingested the highest dose, developed a mixed pattern of liver injury. Median onset of liver injury was 5.5 days.
(Charuwan Sriapha et al., 2020)		• Retrospective study of patients with imidacloprid poisoning who were referred to the Ramathibodi Poison Center in Bangkok, Thailand between 2010 and 2018. The common presenting features were gastrointestinal symptoms (63.8%) with no corrosive injuries and neurological effects (14.2%). The majority of medical outcomes was no (18.4%) to mild (76.1%) toxicity. One patient had symptoms mimicking cholinergic syndrome, three developed liver injury, and five died. Among the five deaths, two patients presented severe initial severity, and one presented moderate initial severity. Two of the patients who died initially presented only mild severity. The mortality rate was 3.1%. The estimated amount of ingestion, cardiovascular effects (especially tachycardia and cardiac arrest), central nervous system effects (especially coma), dyspnoea, and diaphoresis were significantly associated with mortality.
(Lu et al., 2023)		• Study aimed to assess urinary levels of NEOs/metabolites and their associations with children's adiposity measures, and to further investigate the potential role of oxidative stress. A total of nine indicators of adiposity were measured. • 380 children who participated in the 7th year's follow-up of the Laizhou Wan Birth Cohort in northern China. • Six NEOs [imidacloprid (IMI, 99.7%), clothianidin (CLO, 98.9%), dinotefuran (DIN, 97.6%), thiame-thoxam (THM, 95.5%), acetamiprid (ACE, 82.9%), THD (THD, 77.6%)] and two metabolites [N-desmethyl-acetamiprid (N-DMA, 100.0%), 6-chloronicotinic acid (6-CINA, 97.9%)] exhibited high detection rates. • Positive associations of urinary levels of IMI with waist circumference, waist-to-height ratio (WHtR)

Reference	Cited in	Findings
		• Higher IMI levels were associated with overweight/obesity (OR = 1.556, 95% CI: 1.100, 2.201) and abdominal obesity (OR = 1.478, 95% CI: 1.078, 2.026) in children

8.3 Animal findings

Table X.94: Overview toxicokinetics and metabolism in animals

Reference	Cited in	Findings
Kapoor et al. 2014	(2020 NTP, 2020)	• Single dose of imidacloprid (IMI-20mg/kg bodyweight) was orally administered in female rats. • Imidacloprid disposition along with two metabolites 6-chloro nicotinic acid (6-CNA) and 6-hydroxy nicotinic acid (6-HNA) was monitored in organs (brain, liver, kidney, and ovary) and bodily fluids (blood, urine) at 6, 12, 24 and 48 h and faeces at 24 and 48 h. • Maximum concentration (Cmax) of IMI and metabolites in each organ and bodily fluid occurred after 12h. Area under curve (AUC) of IMI ranged from 35 to 358 µg/mL×h; 6-CNA: 27.12-1006.42 µg/mL×h and 6-HNA: 14.98-302.74 µg/mL×h in different organs and bodily fluids. • Clearance rate of IMI was maximum in ovary followed by kidney, liver, brain, faeces, blood and urine. Percent inhibition of acetyl-cholinesterase (AChE) was comparable in brain and Red Blood Cells (RBC) at 6-48 h which suggests the RBC-AChE as valid biomarker for assessing IMI exposure. It is evident that IMI was absorbed, metabolized, and excreted showing increased level of serum enzymes like Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT) and biochemical constituents like bilirubin and Blood Urea Nitrogen (BUN) at 48 h.
Klein 1987a	(FAO and WHO, 2002)	• C14 labelled imidacloprid given to rats (5/sex/dose) as a single dose of 1 mg/kg bw intravenously or as a single dose of 1 or 20 mg/kg bw orally • After sacrifice 48 h after treatment, the concentration of total radiolabel was determined in organs and tissues • After oral administration, the substance was extensively absorbed from the intestinal lumen and readily eliminated • After i.v. administration of 1 mg/kg bw about 92% of the recovered radiolabel was excreted in urine and faeces within 48 h with most of the substance excreted via kidneys with an average ratio urine/faeces of 4:1 • After oral administration about 96% of the dose was excreted in urine and faeces within 48 h with no difference between male and female rats • More than 90% of renal radiolabel was excreted in the first 24 h • Average residual radiolabel in the body, excluding the gi tract, at sacrifice was about 0.5%, and 0.06% of the administered dose in the gi tract • Rats with bile-duct fistulas excreted only 4.7% of the administered dose with faeces, 56% in urine, about 36% with bile, indicating enterohepatic circulation • No significant excretion in exhaled air • Half-lives varied from 2.6-3.6 to 26-118 h

Reference	Cited in	Findings
Klein 1987b	(FAO and WHO, 2002)	• Distribution of C14 imidacloprid investigated in rats by conventional whole-body autoradiography on X-ray film with single doses of 20 mg/kg bw orally given to rats • Distribution was visualized 1, 2, 4, 24, 48 h after treatment • Substance was readily absorbed and rapidly distributed in tissues and organs, readily • Higher concentrations were seen in the thyroid and adrenals, but, after 24 h, all other organs and tissues showed only small amounts of radiolabel • concentrations in the fatty tissues and in the central nervous system were very low throughout the study
Klein & Brauner, 1991	(FAO and WHO, 2002)	• Imidacloprid D4 (imidazolidin-4,4,5,5 D4) was administered orally to male and female rats at a dose of 1 mg/kg bw and additionally to male rats at a dose of 150 mg/kg bw, in a study conducted according to GLP • The stable isotope label was determined in excreta and in plasma as a function of time, and single organs and tissues were assayed for total stable isotope at sacrifice 48 h after treatment • The administered stable isotope was extensively absorbed from the intestinal lumen and rapidly distributed in the body • Excretion was rapid, and the renal route predominated
Klein & Karl 1990	(FAO and WHO, 2002)	• The metabolic pattern of imidacloprid and its nitroso metabolite in excreta was investigated in male rats after administration of a single oral dose of 1 mg/kg bw of imidacloprid or the nitroso compound and after administration of a high dose of 150 mg/kg bw imidacloprid • After the low dose of imidacloprid, about 82% of the renal radiolabel was detected. • main renal metabolite (about 30%) was the glycine conjugate of 6-chloronicotinic acid. The parent compound was present at about 12% • the two monohydroxylated biotransformation products (4-hydroxy and 5-hydroxyimidacloprid) at about 19% and the olefinic metabolite at about 11%. 6-Chloronicotinic acid represented 7.9% of the renal radiolabel • No nitroso compound was formed under these conditions • In the faeces, imidacloprid, the olefinic metabolite, 6-chloronicotinic acid and its glycine conjugate were identified
(Nimako et al., 2021b)		• Detection and quantification of imidacloprid and seven of its metabolites in tissue specimens, and to determine the biodistribution of the IMI compounds in tissues of C57BL/6J male mice (0.6 mg/kg bw/day of IMI in diet for 24 weeks) • Brain, testis, lung, kidney, inguinal white adipose tissue and gonadal white adipose tissue mainly accumulated IMI, blood and mesenteric white adipose tissue mainly accumulated IMI-olefin; liver mainly accumulated desnitroIMI; pancreas predominately accumulated 4-hydroxy-IMI. The desnitro-dehydro-IMI and the desnitro-IMI metabolites recorded tissue-blood concentration ratios ≥ 1.0 for testis, brain, lung and kidney

Reference	Cited in	Findings
(Passoni et al., 2021)		• Mouse dams were treated with doses from 0.118 mg/kg bw day up to 41 mg/kg day between gestational days 6–9. • Levels of imidacloprid in plasma (from 30.48 to 5705 ng/mL) and brain (from 20.48 to 5852 ng/g) of treated mice, proving the passage through the mammalian blood-brain barrier with a high correspondence between doses and measured concentrations • Eight metabolites including imidacloprid-olefin, hydroxy-imidacloprid dihydroxy-imidacloprid, imidacloprid-nitrosimine, desnitro-imidacloprid, 6-chloronicotinic acid, 5-(methylsulfanyl)pyridine-2-carboxylic acid and N-imidazolidin2-ylidenenitramide identified in plasma and brain. Moreover, analysis of embryonic tissues after oral treatment of mouse dams showed detectable levels of imidacloprid (816.6 ng/g after a dose of 4.1 mg/Kg bw day and 5646 ng/g after a dose of 41 mg/Kg bw day) and its metabolites, proving the permeability of the placenta barrier

Table X.9: Overview acute toxicity studies

Reference	Cited in	Route of Exposure	Species	Results (LD50/LC50)
Anonymous 1991a **Boman 1991a**	ECHA reg dossier, CLH opinion (FAO and WHO, 2002)	Oral gavage (OECD 401, GLP)	Rat (Wistar) Purity: 96%	642 mg/kg bw for males, 648 mg/kg bw for females
Anonymous 1989a **Bomann 1989a**	ECHA reg dossier, CLH opinion (FAO and WHO, 2002)	Oral gavage (OECD 401, GLP)	Rat (Wistar) Purity: 94.2%	424 mg/kg bw for males, 450-475 mg/kg for females
Anonymous 1991b **Bomann 1991b**	ECHA reg dossier, CLH opinion (FAO and WHO, 2002)	Oral gavage (OECD 401, GLP)	Rat (Wistar) Purity: 94.3	504 mg/kg bw for males, 379 mg/kg bw for females
Anonymous 2006	CLH opinion	Oral gavage (OECD 423, GLP)	Rat (Wistar) female	Between 300 and 2000 mg/kg bw
Anonymous 1989b **Bomann 1989b**	ECHA reg dossier, CLH opinion (FAO and WHO, 2002)	Oral gavage (OECD 401, GLP)	Mouse (NMRI) Purity: 94.2%	131 mg/kg bw for males, 168 mg/kg bw for females
Anonymous 1987 **Pauluhn 1988a**	ECHA reg dossier (FAO and WHO, 2002)	Inhalation (OECD 403, GLP)	Rat (Wistar) Purity: 95.3%	>0.069 mg/L for males and females
Anonymous 1987 **Pauluhn 1988a**	ECHA reg dossier (FAO and WHO, 2002)	Inhalation (OECD 403, GLP)	Rat (Wistar) Purity: 95.3%	>5.32 mg/L for males and females
Anonymous 1989 **Krötlinger 1989**	ECHA reg dossier (FAO and WHO, 2002)	Dermal (OECD 402, GLP)	Rat (Wistar) Purity: 94.2	>5000 mg/kg bw for males and females
Krötlinger 1990	(FAO and WHO, 2002)	Intraperitoneal	Rat (Wistar) Purity: 94.2%	160-172 mg/kg bw for males

Table X.95: Overview of animal studies with repeated exposure

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Anonymous 1989 **Eiben and Rinke 1989**	ECHA reg dossier (FAO and WHO, 2002)	Wistar Rat (10/sex/dose)	0, 150, 600, 2400 ppm (0, 14.0/20.3, 60.9/83.3, 300.2/422.2 mg/kg bw/d for males and females, respectively)	Oral feed, 96 days, imidacloprid, OECD 408, GLP Purity: 95.3%	NOAEL: 150 ppm (14.0 mg/kg bw/d) in males, 600 ppm (83.3 mg/kg bw/d) in females	- Bw and bw gain decreased in males at 600 ppm, significantly at 2400 ppm in males and females - Food consumption increased at 2400 ppm in males and females - Slightly increased thromboplastin time and decreased thrombocyte counts in males and females at 2400 ppm even after recovery - Sign. Increased AP and ALT activity, in males at 2400 ppm, decreased triglycerides, protein levels and albumin in m and f at 2400 ppm - Abs. Liver and kidney weight decreased in m and f at 2400 ppm, abs. Adrenal glands in f at 2400 ppm, increased rel. Brain weights in m and f, spleen in f, testes in m - No gross pathological findings - Histopathologically, slight changes in plasma of hepatocytes in m at 600 ppm, round cell infiltrates in liver, isolated cell necrosis in liver, focal necrosis, higher incidence of cytoplasmic changes and swollen liver cell nuclei in males at 2400 ppm, effects were reversible
Anonymous 1988 **Ruf 1990**	ECHA reg dossier (FAO and WHO, 2002)	Beagle dog (4/sex/dose)	0, 200, 600, 1800/1200 ppm (0, 7.8, 23.0, 42.0 mg/kg bw/d for males and females) 1800 ppm decreased to 1200 ppm after 4 weeks due to low food consumption	Oral feed, 90 days, imidacloprid, OECD 409, GLP Purity: 95.3%	NOAEL: 200 ppm (7.8 mg/kg bw/d) for males and females	- Trembling in all animals from 600 ppm, severe trembling at 1800 ppm, no trembling after reduction to 1200 ppm - Bw gain decreased at 1800 ppm, normal after reduction to 1200 ppm - Food consumption decreased at 1200/1800 ppm, to a lower extent at 600 ppm - Ophthalmological transient slight corneal erosion from 600 ppm in m - Slight haematological, clinical chemistry and urinalysis changes independent of dosage - Some changes in organ weights but all within HCD - At gross pathology tonsils reddening without dose dependency - No histopathological findings

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Bloch et al. 1987	(FAO and WHO, 2002)	Beagle dog (2/sex/dose)	0, 200, 1000, 5000 ppm (0, 7.3, 31, 49 mg/kg bw/d for males and females combined)	Oral feed, 28 days, GLP Purity 92.8%	NOAEL. 200 ppm (7.3 mg/kg bw/d)	- 2 HD animals died, the other 2 were killed in moribund condition with ataxia, tremor, occasional vomiting - Decreased food consumption in MD and HD animals - Markedly decreased bw in HD animals - CYP activity in liver slightly increased only in MD animals - Increased liver weight only in one MD female
Anonymous 1990 Eiben, 1991 Eiben and Kaliner, 1991	ECHA reg dossier (FAO and WHO, 2002)	Wistar Rat (50/sex/dose)	0, 100, 300, 900, 1800 ppm (5.7/7.6, 16.9/24.9, 51.3/73.0, 102.6/143.7 mg/kg bw/d for males and females, respectively)	Oral feed, 24 months, imidacloprid, OECD 453, GLP Purity: 94.3-95.3%	NOAEL: 100 ppm (5.7 mg/kg bw/d) for males, 300 ppm (24.9 mg/kg bw/d) for females	- Bw gain decreased from 900 ppm dose dependently - Decreased water intake in HD females - AST sign. Increased HD m and f, creatine kinase increased in HD f - Decreased abs. and re. Organ weights in HD considered secondary to decreased bw - Mineralized particles in the thyroid glands of males of all dose groups with a dose dependent increase, in females only in top dose (final section: 2/50 (control), 12/50 (100 ppm), 31/50 (300 ppm) and 44/50 (900 ppm) in male rats and 11/50 (control), 6/50 (100 ppm), 11/50 (300 ppm) and 27/50 (900 ppm) in female rats) - Plasma levels of thyrotropin, triiodothyronine and thyroxine were unaffected
Anonymous 1988 **Allen et al. 1989**	ECHA reg dossier (FAO and WHO, 2002)	Beagle Dog (4/sex/dose)	0, 200, 500, 1250/2500 ppm (0, 6.1, 15, 41/72 mg/kg bw/d for males and females) Top dose increased from week 17 onwards	Oral feed, 52 weeks, imidacloprid, OECD 452, GLP Purity: 94.9%	NOAEL: 500 ppm (15 mg/kg bw/d)	- No increased mortality - Decreased food consumption in HD animals in first week and after increasing dose - Cholesterol sign. Increased in HD females, liver cyp levels sign. Increased in HD males and females - Relative brain weight slightly increased in HD animals, considered as adaptive process due to induced metabolism - No gross pathological or histopathological findings

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Anonymous 1988 **Pauluhn 1988d**	ECHA reg dossier (FAO and WHO, 2002)	Wistar rat (10/sex/dose)	0, 5.5, 30, 190 mg/m^3	Inhalation (nose only), 28 days, imidacloprid, OECD 412, GLP	NOAEC: 5.5 mg/m^3	- No increased mortality - Sign. Decreased bw in HD males - Hquick. Blood coagulation time, total serum bilirubin increased in HD females, decreased thrombocyte count in HD males - Alt, ap, n-demethylase activity increased, che decreased in md females, a-globulin levels decreased in MD m and f - Alt, ap increased in HD f, gldh increased in HD m+f, increased bilirubin in HD f, decreased triglyceride in HD m and f, decreased che in HD f, increased o-demethylase and n-demethylase in HD m and f, increased cyp in HD m, decreased a-globulin in HD m and f - Sign higher ph in urinalysis in HD f - Sign. Increased rel. Liver weight in md and HD f - Rel. Heart and thymus weight slightly decreased in HD f, rel. Brain and heart weight slightly decreased in HD m but within hcd - Sign. Increased proerythroblasts in bone marrow of all treated m, not sign. In f, segmented neutrophils decreased in m and f of all treated groups
Pauluhn 1988a	(FAO and WHO, 2002)	Wistar rats (10/sex/dose)	0, 20, 110 and 500 mg/m^3	Inhalation (nose only), 5 days, GLP Purity: 95.3%	NOAEC: 20 mg/m^3	- Bw gain slightly decreased ant MD and HD - Increased O-demethylase and N-demethylase activities in liver homogenates from MD animals
Anonymous 1988 **Flucke 1990**	ECHA reg dossier (FAO and WHO, 2002)	New Zealand White rabbit (5/sex/dose)	0, 1000 mg/kg bw/d	Dermal semiocclusive, 15 days, imidacloprid, OECD 410, GLP Purity: 95%	NOAEL: 1000	- No treatment related increased mortality - Transient increased food consumption in the first week in all treated animals - Slightly increased abs. And rel. Brain weights, slightly decreased ovary weights considered incidental

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Anonymous 1990 Watta-Gebert, 1991a,b	ECHA reg dossier (FAO and WHO, 2002)	B6C3F1 mouse (50/sex/dose)	0, 100, 330, 1000, 2000 ppm (0, 20.2/30.3, 65.6/103.6, 208.2/274.4, 413.5/423.9 mg/kg bw/d for males and females, respectively)	Oral feed, 24 months, OECD 451, GLP Purity: 95.3%	NOAEL: 330 ppm (65.6/103.6 mg/kg bw/d in males and females, respectively)	- "squeaking and twittering" type of vocalization in HD animals - No treatment related increased mortality - Bw and Bw gain decreased in MD males, sign. In HD males - Food consumption decreased in MD females, sign. In HD females - Water intake decreased dose dependently in MD and HD animals - Depressed leukocyte counts in HD animals - Increased AP, decreased cholesterol in HD animals - Abs. Lung, liver, kidney, adrenals, brain, ovary weights sign. Decreased - "minor" gross pathological findings such as discolorations and changes in size and shape of several organs, cystic and enlarged ovaries, collapsed lungs, hair loss in skin and nodes in several organs - Slight periacinary liver cell hypertrophy and loss of vacuolation in the tubules of kidneys in HD males - Increased incidence of mineralization in the brain (thalamus) in HD animals - Greatly decreased incidences of tubular basophilia in HD males and of lymphocytosis in the renal pelvis in HD males and females might be secondary to decreased food consumption
Eiben 1988a	(FAO and WHO, 2002)	B6C3F$_1$ mice (10/sex/dose)	0, 120, 600, 3000 ppm (0, 17, 86, 430 mg/kg bw/d)	Oral feed, 107 days Purity: 92.8%	NOAEL. 120 ppm (17 mg/kg bw/d)	- 7 HD males + 7 HD females died - Poor general condition in several animals - Decreased bw gain and food consumption in MD and HD males and HD females - Sign. Decreased urea and cholesterol in HD males, decreased ALAT and glucose in HD females - AP sign. Increased HD males and in all treated females - Differences in liver, heart, spleen, kidneys, testes and adrenals weights in HD animals

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Eiben 1988b	(FAO and WHO, 2002)	Wistar rat (10/sex/dose)	0, 120, 600, 3000 ppm (0, 11, 57, 410 mg/kg bw/d for males and 15, 78, 510 mg/kg bw/ for females)	Oral feed, 98 days Purity: 95.3%	NOAEL. 120 ppm (11 mg/kg bw/d)	- Food intake increased in all HD animals, bw gain decreased at MD and HD - Sign. Increased AP sign. Decreased glucose in HD animals, decreased cholesterol in HD males - Degenerative histological lesions in the epithelium of the testicular tubules and multifocal group cell necrosis in liver of HD males
Sun 2016	(2020 NTP, 2020)	Male C57BL/6J mice	0.06, 0.6, 6 (mixed in a low-fat (4% w/w) or high-fat (20% w/w) diet)	Gavage, 12 weeks		- Significantly promoted high fat diet-induced body weight gain and adiposity - Imidacloprid treatment with the high fat diet resulted in impaired glucose metabolism, effects on genes regulating lipid and glucose metabolisms, including the amp-activated protein kinase-alpha (ampkalpha) pathway in white adipose tissue and liver
Pandit 2016	(2020 NTP, 2020)	Mice (6/group)	1/20(th) of LD50 dissolved in corn oil	Gavage (in corn oil), 30 days		- Effects of imidacloprid alone or in combination with microbial molecules on lungs - There was no change in the expression of tlr-4 and tnf-alpha both at mRNA and protein levels following exposure to imidacloprid
Toor 2013	(2020 NTP, 2020)	Female albino rat	1/10th and 1/50th of LD50 of imidacloprid	Oral		- Histological examination of liver revealed that imidacloprid low dose treatment resulted in dilations of central vein and sinusoids between hepatocytes however high dose did not induce histopathological changes in liver. - Non-significant decrease in alkaline phosphatase (ALP) activity observed - Liver aspartate aminotransferase level showed significant increase in higher dose of imidacloprid. - Additionally, significant increases in plasma levels of aspatate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were observed in the treated rats.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Soujanya 2013	(2020 NTP, 2020)	Sprague Dawley rats (12/group)	80	Oral, 28 days		- The effects of oral administration of imidacloprid for 4 weeks on serum biochemical, oxidative stress, histopathological and ultrastructural alterations - The serum biochemical assays revealed a significant in alanine transaminase and aspartate transaminase and decrease in total protein - The tissue biochemical profile revealed a significant reduction in reduced glutathione concentration in the liver - Histologically, the liver showed marked dilation, congestion of central vein, portal vein and sinusoidal spaces, vacuolation/fatty change and degenerated hepatocytes. Ultra-thin sections of the liver revealed swollen nuclei, varied size and shape of mitochondria, disrupted chromatin and rough endoplasmic reticulum
Badgujar 2013	(2020 NTP, 2020)	Female BALB/c mice	2.5, 5, 10	Oral, 28 days	2.5	- Objective of the study was to evaluate immunotoxic effects of imidacloprid - Imidacloprid at high dose suppressed cell-mediated immune response as was evident from decreased DTH (delayed type hypersensitivity) response and decreased stimulation index of T-lymphocytes to PHA. - At this dose, there were also prominent histopathological alterations in spleen and liver. Histopathological analysis of footpad sections of mice revealed dose-related suppression of DTH response. - Imidacloprid at low dose of 2.5mg/kg/day did not produce any significant alterations in cellular and humoral immune response - Immunosuppressive effects at doses >5mg/kg, which could potentially be attributed to direct cytotoxic effects of IMD against T cells (particularly TH cells)
Mzid 2018	(2020 NTP, 2020)	Female rat	50, 200, 300	60 days		- Evaluate the protective effect of UU (urtica urens) against the toxicity of Confidor((R)), whose active substance is imidacloprid (IMI)

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- IMI caused an acute ovary injury, increased the ovary tissue levels of MDA and AOPP, and decreased the levels of GSH, vitamin E, and antioxidant enzyme activities. - The number and the diameter of follicles were markedly diminished together with a reduction of the relative weight of ovaries - Significant reduction in serum 17b-estradiol levels
Mzid 2017	(2020 NTP, 2020)	Female rat	50, 200, 300	60 days		- Evaluate the protective effect of UU (urtica urens) against the toxicity of imidacloprid (IMI) - Significant decreases in cortical, trabecular thicknesses and osteoid surfaces. - Significantly decreased serum vitamin D and hydroxyproline levels - Increased significantly calcium, phosphorus contents, MDA and AOPP levels and the rate of calcification. - It decreased significantly GSH, gpx, SOD, CAT, 17b-Estradiol and vitamin E levels, induces a tendency of rarefaction and increases of intrabecular spaces
Lafi 2018	(2020 NTP, 2020)	Rats	64	Oral via drinking water, 3 weeks		- Investigated the effects of IMI and gibberellic acid GA3 separately or together on rats kidney - Increased the levels of kidney malondialdehyde, advanced oxidation protein products, protein carbonyls and metallothionein, plasma creatinine, urea, blood urea nitrogen and lactate dehydrogenase activity. A decline of kidney uric acid level and antioxidant status was also observed. All these changes were supported by histopathological observations
Arfat 2014	(2020 NTP, 2020)	Male albino mice (12/group)	5, 10, 15			- Decrease in the body weight of the high dose group - No mortality occurred during the treatment period - High dose of imidacloprid caused a significant elevation of serum clinical chemistry parameters, serum glutamic oxaloacetic

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TBIL). - Histology of liver and kidney indicates hepatotoxicity and nephrotoxicity at a high dose of imidacloprid.
Mohany 2012	(2020 NTP, 2020)	Male albino rats	0.21	Oral, 28 days		- Aim of the study was to investigate the impact of thymoquinone (TQ) on IMI-induced immunotoxicity - IMI induced significant increases in total leukocyte counts, total immunoglobulins (IGs) (especially IGGs), the hemagglutination of antibodies, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and malondialdehyde (MDA) compared to the control group. - Significant decreases in phagocytic activity, chemokine expression and chemotaxis were observed in the IMI-treated group, as were severe histopathological lesions in the liver, spleen and thymus
(Ozsahin et al., 2014)	(X. Zhang et al., 2023)	Infant and adult male rats (7/group)	4	Oral, 90 days		- Changes in kidney biochemical parameters which seem to be dependent on the pesticide and age of the animal (infant or adult)
(Abdelhafez et al., 2022)		Adult male albino rats (5/group)	0, 22.5, 45	Oral gavage, 28 days		- The aim of the study was to assess oxidative stress in liver cells and genotoxicity of erythrocytes for rats exposed to sub-lethal doses of IMI and the protective effects for Rhodophyta - Significant increase in lipid peroxidation and reductions in glutathione were observed in liver cells at high and low doses of IMI (22.5, 45) - Decreases in catalase and glutathione peroxidase parameters indicating oxidative stress at all doses. - Significant increases in micronucleus frequency (MN) in the bone marrow of the rats as a genotoxicity marker which indicated DNA damage in erythrocytes cells with alterations in the histopathology of liver cells were also noted such as necrosis, inflammatory cells, infiltration, and necrobiotic changes. Effect occurred at all doses.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Zhang et al., 2023)		Male C57/BL6 mice (8/group)	0, 1.67	Orally, 30 days		- The mitochondrial crest of kidney cells significantly decreased following IMI exposure. Moreover, IMI exposure triggered ferroptosis and lipid peroxidation in the kidney. - Nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated antioxidant capability was negatively correlated with the ferroptosis induced by IMI exposure. - Induced F4/80+ macrophages to accumulate in the proximal tubules of the kidneys, and also increased the protein expression of high-mobility group box 1 (HMGB1), receptor for advanced glycation end products (RAGE), receptor for advanced glycation end products (TLR4), and nuclear factor kappa-B (NF-κb) - IMI stress can induce Nrf2 inactivation, thereby triggering ferroptosis, causing an initial wave of death, and activating HMGB1-RAGE/ TLR4 signalling, which promotes pyroptosis that perpetuates kidney dysfunction.
(Abd-Elhakim et al., 2023)		Male Sprague-Dawley rats (10 rats/group)	0, 22.5	Orally in corn oil, 56 days		- The possible beneficial effect of thymol (TML) in relieving IMID-induced harmful effects on the brain - IMID-induced depletion of the total antioxidant capacity of the brain tissues - Decrease in gamma-aminobutyric acid but the increase in acetylcholinesterase - organic brain DNA damage
(Akter et al., 2023)		Adult male rabbits (6/group)	0, 0.5 ml (100 mg)/L water	Orally (contaminated green grass), 15 days		- The residual effects of exposure to IMI-contaminated diet on liver, lung, heart, and kidney - The levels of hepatic serum aspartate transaminase and alanine transaminase were considerably elevated in IMI-exposed rabbits - The residue of IMI was at the detectable level in the liver and stomach. - The liver revealed coagulation necrosis with granulomatous inflammation and congestion in portal areas with dilated and congested central veins.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- The lungs showed congestion of blood vessels and granulomatous inflammation around the terminal bronchiole. - Accumulations of inflammatory cells were observed in the cortico-medullary junction in the kidney. - The heart showed necrosis and infiltration of mononuclear cells within the cardiac muscles
(Askri et al., 2022)		Adult male Wistar rats (10/group)	0, 25	Orally, 30 days		- Study investigated the potential effects on hepatic function of Imidacloprid and Deltamethrin in rats - Decrease in relative liver weights; hepatic activity was altered - A significant rise at the level of hepatic enzymes, blood sugar and lipids versus decreased protein in treated rats. These modifications were accompanied by alterations of the hepatic tissue structure in the treated rats, compared to the controls which remained intact
(Farag et al., 2020)		Mature male rats (10/group)	0, 0.21	Orally Using rat stomach tube, 3 months		- Evaluation of the hepatotoxic effect of Imidacloprid (IMI) in rats and assessment the modulatory role of Zingiber officinale Roscoe, ginger aqueous extract (GAE), against such effect - Significant increase in alanine aminotransferase (ALT), Aspartate amino transferase (AST) levels, significant decrease in total proteins, albumin and globulins in serum - IMI upregulates interleukin 6 (IL6), tumor necrosis factor- α (TNF-α) and toll like receptor 2 (TLR2) genes in the liver of rats associated with intense immuno positive reactivity of TLR2 and TNF α in the liver of IMI-treated group - Significant alterations in the liver tissue of IMI-treated group, such as disorganization of hepatic cords replaced by mononuclear cells which surrounded by lymphocytes and mild congestion of blood vessels was also seen
(Haddad et al., 2023b)			0, 1.2	Orally, 90 days		- Effect of chronic exposure to imidacloprid (IMI) on the neurotoxicity and protective role of catechins

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- Significant decreases in mitochondrial glutathione (GSH) rate and catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (gpx) activity, while glutathione-S-transferase (GST) activity Malondialdehyde (MDA) and cytosolic calcium (Ca2+) levels were significantly increased. The same results showed an improvement in mitochondrial membrane permeability as well as an increase in mitochondrial oedema. - Results showed that chronic exposure to IMID is associated with loss of mitochondrial integrity, dysfunction of the cholinergic system, and impairment in neurobehavior and cognitive ability
(Hassanen et al., 2022)		Male albino Wistar rats (7/group)	0, 45	Orally, 28 days		- Study to explore the mechanism of each IM and HFM–evoked rat liver and kidney toxicity - Changes in walking, body tension, alertness, and head movement with a significant reduction in rats' body weight - Significant increases in MDA levels and decrease of GHS levels were recorded in liver and kidney homogenates - Extensive histopathological alterations with a significant increase in the serum levels of ALT, AST, urea, and creatinine and a decrease in total proteins, albumin, and globulin levels in liver and kidney tissues - Upregulation of the transcript levels of casp-3, JNK, and HO-1 genes with strong immunopositivity of casp-3, TNF-ά, and NF-KB protein expressions in the liver and kidneys
(Katić et al., 2021)		Male Wistar rat (5/group)	0, 0.06, 0.8, 2.25	Orally, 28 days		- Effect of IMI on cholinesterase activity, oxidative stress responses and primary DNA damage in the blood and brain tissue - IMI did not cause significant changes in total cholinesterase, acetylcholinesterase and butyrylcholinesterase activities in plasma and brain tissue - Reactive oxygen species levels and lipid peroxidation increased significantly in the plasma of rats treated with the lowest dose

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- Activities of glutathione-peroxidase in plasma and brain and superoxide dismutase in erythrocytes increased significantly at the highest applied dose - Significant peripheral blood leukocyte damage at the lowest dose of imidacloprid and dose dependent brain cell DNA
(Khayal et al., 2022)		Adult male albino rats (10/group)	22.5	Orally, 8 weeks		- Study investigating protective role of marjoram extract on the immunotoxic response and oxidative stress - Significantly elevated total leukocytes, neutrophils percentage, increased immunoglobulin G and the significantly reduction of lymphocytes percentage, phagocytic activity, phagocytic index and lysozyme activity
(J Li et al., 2023)		Male ICR-strain mice (4/group)	10	Orally, 4 weeks		- Study tested the combined toxicity of microplastics and pesticides - Lowered the body weight gain - Single exposure of IMI induced the tissue damage in liver by disturbing the redox homeostasis
(C Nimako et al., 2021)		C57bl/6j Mice (4/group)	0.6	Orally, 24 weeks		- The mechanistic role of imidacloprid in the prevalence of high fat diet (HFD)-induced liver steatosis - Imidacloprid treatment significantly potentiated HFD-induced body weight gain in mice - Increased the liver weights with complimentary reductions in mesenteric and gonadal white adipose tissue weights. - Histopathological analysis of liver revealed a drastic steatosis. - Imidacloprid upregulated transcriptions of hepatic fatty acid biosynthesis-related transcription factors and genes. Imidacloprid also induced hepatic expression of the gene encoding pregnane X receptor; but had no significant effect on hepatic expressions - Of liver X receptor and aryl hydrocarbon receptor. The imidacloprid treatment further enhanced

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- Serum alanine aminotransferase levels but downregulated hepatic antioxidant mRNA expressions
(Omar et al., 2023)		Male Albino rats (5/group)	0, 14, 28, 54	Orally in drinking water, 28 days	BMD body weight 5.885 BMD liver weight 42.29	- Pomegranate-extract-loaded clove-oil-based nano emulsion (PELCN) and its potential for reducing oxidative stress and DNA damage, as well as its hepatoprotective effects against imidacloprid. - Reduction in body weight gain and hepatotoxic effects as evidenced by increases in the liver enzymes AST, ALT, and ALP. They caused oxidative damage in the liver of male rats as indicated by the decreased liver activity of the GST, GPX, SOD, and CAT enzymes and decreased serum TAC. IM and CPF produced a significant dose-dependent increase in DNA damage in hepatocyte cells, resulting in moderate to severe liver damage with cells that are more inflammatory and have enlarged sinusoids and compacted nuclei.
(Sen et al., 2022)		Female Car−/− and wild-type (WT) mice	50	Orally (twice daily), 21 days		- This study examined the role of CAR (constitutive androstane receptor) in mediating IMI off-target toxicity. - Liver tissue analysis indicated mild inflammation and induction of detoxification gene Cyp2b10 in IMI-exposed WT mice - Microbiome analysis of ileal samples revealed IMI altered microbial diversity in a genotype-specific manner, with increased α-diversity in Car−/− mice while decreased α-diversity in WT mice - IMI is not overtly toxic
(Sevim et al., 2021)		Sprague–Dawley rats (12/group)	0, 5.7	Orally, 90 days		- Study investigating the protective effects of probiotics - IMI induced liver, kidney, brain and bowel damage
(Shao et al., 2020)		Male mice (10/group)	0, 5	Orally, 14 days		- Study investigating protective effect of CAPE (Caffeic acid phenethyl ester) against IMI induced liver injury - Serum AST and ALT increase - Upregulated liver NO generation and lipid peroxidation, and downregulated glutathione, catalase, superoxide dismutase and glutathione peroxidase in a dose-dependent manner in liver

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- Endoplasmic reticulum stress represented by the swelling of endoplasmic reticulum was observed by transmission electron microscope in IMI group - Upregulation of TNF-α and IFN-γ induced by IMI in liver of mice
(Taha et al., 2021)		Male albino rats	0, 9	Orally,28 days		- Multiple pesticides tested - Mitochondrial dysfunction confirmed by a significant decrease in NADH dehydrogenase and ATPase activities. Oxidative stress biomarkers include malondialdehyde (MDA), and protein carbonyl content (PCC) were significantly increased. However, superoxide dismutase (SOD) and glutathione S-transferase (GST) as antioxidant enzymes were significantly decreased in the mitochondria
(Wang et al., 2023)		C57BL/6 mice (6/group)	0, 15	Orally, 7 days		- Toxicity of imidacloprid and cadmium to mammals was investigated - Changes in terms of oxidative stress, abnormalities in lipid metabolism, and disturbances in amino acid metabolism
(Yang et al., 2020)		Male C57BL/6J mice (10/group)	0, 3, 10, 30 mg/l (0, 0.5, 1.67, 5 mg/kg bw/d)	Orally, drinking water, 70 days		- The study showed that exposure to IMI could reduce relative liver weights, change hepatic tissue morphology and induce hepatic oxidative stress. The gut barrier function was greatly impaired by IMI exposure, which might increase the body's susceptibility to harmful substances in the gut. Meanwhile, the synthesis and metabolism of hepatic bile acids (bas) was also affected by IMI exposure. The levels of serum and hepatic total bile acids (tbas) decreased - Causing gut microbiota imbalance
(G.-P. Zhao et al., 2021)		Male Wistar rats (10/group)	0, 0.06	Orally, 90 days		- Significantly increased intestinal permeability, which led to significantly elevated serum levels of endotoxin and inflammatory biomarkers (tumor necrosis factor-alpha and interleukin-1 beta) without any variation in body weight.
(Zheng et al., 2020)		Female KM mice	0, 5, 20	Orally, 28 days		- Damages to the hippocampus and liver of mice in the high-dose group. - Significant differences among the control group, the low-dose group and the high-dose group. The metabolic disturbance in the hippocampus and liver of mice had been induced by low-dose of IMI,

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						although no significant histopathological changes were observed in the low-dose group. - Six differential metabolites in the hippocampus and 10 differential metabolites in the liver were identified. - The metabolism disturbances of important biochemical pathways in the hippocampus and liver of mice in the exposed groups were elucidated, mostly concentrated in lipid metabolism, amino acid metabolism, nucleotide metabolism, carbohydrate metabolism, and energy metabolism.

Table X.96: Overview neurotoxicity studies

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Khalil 2017	(Ongono et al., 2020b)	Male Sprague - Dawley rats (6/group)	0.5, 1	Dietary for 60 days, Imidacloprid (purity grade: 100%)		- Anxiety-like behavior and locomotor activity using the open field test. Depression-like behavior using the forced swim test. Motor coordination and sense of equilibrium by observing the mice's swimming performances. - No differences in swimming performances between groups. - Reduction in the frequency of entries and time spent in the central arena (indicative of anxiety) in the open field test, as well as a substantial increase of grooming in the high-dose group. - Increased immobility time and decline in swimming time - Depression-like behavior in both exposed groups. - Induced anxiety- and depression-like behaviours in young rats
Kara 2015	(2020 NTP, 2020; Ongono et al., 2020b)	Albino Wistar Rats (-for infant model: newborn Males (6/group) -for adult model: 24 male rats 8 to 9-week-old (6/group)	0.5, 2, 8	Dietary for 3 months, Imidacloprid		- Investigating Spatial learning and consolidation of memory using The Morris water maze learning performance and probe trial tests. - Significantly decreased learning skills and memory consolidation
Anonymous 1992	ECHA reg dossier	SD rats (12/sex/dose) (EPA 540/09-91-123, GLP)	0, 20, 50, 150, 350	Oral gavage, Single exposure, 15 days observation	NOAEL overall toxicity: 50	- Functional observational battery prior to treatment and after - 4/18 hd males (2 in main, 2 in satellite group) and 10/18 hd females (8 in main, 2 in satellite group) died on day of administration or the day after - One animal at 150 mg/kg showed tremors and red nasal stain, at 300 mg/kg 9/2 males/females showed tremors, 7/3 had red nasal stain, 8/5 with uncoordinated gate, 7/5 with decreased activity, 4/4 with cool body, 2 females with increased activity - Bw gain slightly increased in surviving hd males

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- Increased haemoglobin, haematocrit mature neurophilia and a lymphopenia in hd females - Decreased serum triglycerides in males at 150 mg/kg, at 300 mg/kg decreased serum triglycerides and alt in m and f, decreased serum potassium and cholesterol in f
Anonymous 1993 **Sheets 1994b**	ECHA reg dossier (FAO and WHO, 2002)	F344 rats (12/sex/dose) (EPA 540/09-91-123)	0, 150, 1000, 3000 ppm (0, 10.5/9.3, 69.5/63.3, 213/196 mg/kg bw/d for males and females, respectively)	Oral feed, 90 days	NOAEL overall: 150 ppm (9,3 and 10.5 mg/kg bw/d in males and females, respectively) Neurotox 3000 ppm	- Functional observational battery prior to treatment and after - Decreased bw and food consumption in MD and HD males and females - Changes in clinical chemistry in all treated animals - FOB in HD: slightly uncoordinated grip strength in m, slightly increased motor and locomotor activity in m, lower motor and locomotor activity in females considered incidental due to lack of significance
Anonymous 1999	ECHA reg dossier	Wistar rats (25-30 P males, 25-20 P females ca 80 F1 males and 80 F1 females) (EPA OPPTS 870.6300)	0, 100 ppm (8.0-8.3 mg/kg bw/d during gestation, 12.8-19.5 during lactation), 250 ppm (19.4-19.7 mg/kg bw/d during gestation, 30.0-45.4 during lactation), 700 ppm (54.7-58.4 mg/kg bw/d during gestation, 80.4-155.0 during lactation)	Oral feed, GD0-LD21		- No increased mortality - Maternal bw and bw gain unaffected - Bw, bw gain and food consumption of HD pups decreased - Reduced motor activity (31-38% on PND 17 for males and females, 37% on PND 21 for females), reduced locomotor activity (37-39% on PND 17 for males and females, 26% on PND 21 for females), no effects were seen after PND 21 but effects were non-significant and reversible - Slight but significant decrease in caudate-putamen width was noted at study termination in HD females - No treatment related reproductive effects
Annabi et al. 2015	(2020 NTP, 2020)	Male rats	0, 40	Intragastric intubation, 28 days		- Study evaluating the recovery effect of NAC against Imidacloprid-induced oxidative stress and cholinergic transmission alteration in hypothalamic-pituitary-adrenal (HPA) axis

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- Imidacloprid increased adrenal relative weight and cholesterol level indicating an adaptive stage of the general alarm reaction to stress. - Significant increase in malondialdehyde level, the antioxidants catalase, superoxide dismutase and glutathione-S-transferase showed various alterations following administration and significant depleted thiols content was only recorded in hypothalamic tissue. - The hypothalamic and pituitary acetylcholinesterase activity and calcium level were significantly increased highlighting the alteration of cholinergic activity.
Bhardwaj et al. 2010	(2020 NTP, 2020)	Female rats	0, 5, 10, 20	Oral, 90 days	10 (NOEL)	- Decrease in the body weight gain was observed at 20mg/kg/day and at necropsy the relative body weights of liver, kidney and adrenal was also significantly increased at this dose level. - No mortality occurred during treatment period while food intake was reduced at high dose level. - In clinical chemistry parameters high dose of imidacloprid has caused significant elevation of serum GOT, GPT, glucose and BUN and decreased the activity of ache in serum and brain. - The spontaneous locomotor activity was also decreased at highest dose exposure as there were no significant changes in haematological and urine parameters. The brain, liver and kidney of rats exposed with high dose of imidacloprid had showed mild pathological changes. - Based on the morphological, biochemical, haematological and neuropathological studies it is evident that imidacloprid has not produced any significant effects at 5 and 10mg/kg/day doses but induced toxicological effects at 20mg/kg/day to female rats.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
Duzguner and Erdogan et al. 2012	(2020 NTP, 2020)	Wistar rats (n = 10 rat/each group).	1	Orally by gavage for 30 days		- Study investigating inflammatory responses to chronic exposure of imidacloprid - Imidacloprid application caused a significant increase in nitric oxide production in brain and liver. - Induced the mRNA transcriptions of the three isoforms of nitric oxide synthases (inos, enos, nnos) in brain and two isoforms (inos, enos) in the liver. - Significant lipid peroxidation in plasma, brain and liver. While the superoxide-generating enzyme xanthine oxidase activity was elevated in both tissues, myeloperoxidase activity was increased only in the liver. - Oxidative stress and inflammation by altering antioxidant systems and inducing pro-inflammatory cytokine production in the liver and central nervous system of non-target organisms.
Freeborn et al. 2015	(2020 NTP, 2020)	Rat	0, 50, 100	Oral gavage		- Changes in the EEG (electroencephalogram) - Imidacloprid did not alter the EEG
Kapoor et al. 2010	(2020 NTP, 2020)	Female Rat	0, 5, 10, 20	Oral gavage, 90 days	10 (NOEL)	- Effect on oxidative stress and Lipid peroxidation (LPO) - Imidacloprid at 5 and 10 mg/kg/day has not produced changes in SOD, CAT, gpx and level of GSH and LPO in liver, brain and kidney. - However, 20 mg/kg/day has produced significant changes in SOD, CAT, gpx, GSH, LPO in liver; SOD, CAT and gpx in brain and LPO in kidney. - NOEL through antioxidant enzymes and LPO in female rats.
Khalil et al. 2017	(2020 NTP, 2020)	Rat	0, 0.5, 1	Orally, 60 days		- Evaluated the stress response in adult rats - It led to an alteration in the levels of cortisone and catecholamines and induced behavioral deficits, particularly in the animals exposed to the dose of 1.0mg/kg.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- IMI was further analyzed for the effect on glucose homeostasis in developing and adult rats at a dose of 1.0mg/kg bw where it elicited a hyperglycaemic effect. - Alteration in the mRNA levels of glucose transporters. - histopathological and immunohistochemical data displayed structural perturbations in pancreatic tissue with a decline in the expression of insulin and glut4, particularly in the developing rats. - Collectively, IMI treatment resulted in stress represented by behavioral and biochemical changes, particularly at a dose of 1.0mg/kg bw.
Lonare et al. 2014	(2020 NTP, 2020)	Rat	0, 45, 90	Orally, 28 days		- The neurotoxic effect and biochemical alteration as a result of imidacloprid (IMI) exposure and potential protective role of curcumin (Cur) against it in rats. - Significant decrease in spontaneous locomotor activity (SLA) and pain threshold were observed in animals treated with the IMI - Acetylcholinesterase, ATPase and serum biochemicals such as creatine kinase, lactate dehydrogenase, sorbitol dehydrogenase and alkaline phosphatase levels were significantly decreased as result of IMI exposure. - A significant decrease in antioxidant enzymes activity and non-enzymes level with increase in lipid peroxidation (LPO)
Nellore et al. 2010	(2020 NTP, 2020)	Albino rat	0, 400	Oral gavage		- The effect of imidacloprid on ache enzyme system in different regions like Striatum, Hippocampus, Medulla, Cerebral cortex and cerebellum of brain - Ache Levels were significantly decreased in toxin exposed rats in all regions of the brain when compared to control rats. Contrast to ache levels ach levels was significantly elevated.
Vohra et al. 2014	(2020 NTP, 2020)	Albino rat	0, 10, 20	Oral, 60 days		- Average feed intake was significantly reduced at 20mg/kg/day. - Relative weight of heart and spleen decreased significantly at higher dose level.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- Non-significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) activity was observed in both the imidacloprid treated groups - Significant decrease in acetyl cholinesterase (ache) activity in plasma and brain of both the imidacloprid treated groups. Microscopically, liver tissue of rats treated with higher dose of imidacloprid showed marked dilation and congestion of central vein and degeneration of hepatocytes. The exposure to imidacloprid produced histopathological changes that could be correlated with changes in the biochemical profile of female albino rats. The blood plasma proteins were examined by SDS PAGE. There was no diagnostic difference in the pattern of plasma protein profile of control and treated rats. - Based on the present physiological, biochemical and histological studies it is evident that imidacloprid did not produce any significant effects at 10mg/kg/day dose but induced toxicological effects at 20mg/kg/day to female rats.
Sheets 1994a	(EFSA Panel on Plant Protection Products and their Residues (PPR), 2013; FAO and WHO, 2002)	Sprague-Dawley [Sas:CD(SD)BR] rats (18/sex/group)	0, 42, 150, 310	Oral gavage, single dose Purity: 97.6-98.8%	42	- 4 HD males and 10 HD females died on day of treatment or day after - Dose-dependent increase of clinical signs with treatment-related effects in MD (tremors and nasal staining) and HD (also uncoordinated gait, decreased activity and urine staining and were cool to touch) males and in HD females (tremors, uncoordinated gait, decreased activity, increased reactivity and red nasal staining) - FOB effects in MD and HD males and females: increased incidence of recumbency - Dose-related decrease in a measure of motor and locomotor activity in all treated females and MD and HD males on day 0 - Decreased serum triglyceride concentration in MD animals

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- Decreased serum potassium and cholesterol in surviving HD females, decreased ALAT in HD males and females - Haematological changes in HD males and females
(Hassanen et al., 2023)		Male Wistar rats (7/group)	0, 45	Orally, 60 days		- Study investigating The potential neuroprotective impact of both OMO (Origanum majorana oil) and OME (methanol extract) against IMI-induced neurotoxicity - Neurobehavioral and neuropathological alterations and a reduction of both acetylcholine esterase (ache) activity and dopamine (DA), serotonin (5HT), and γ-aminobutyric acid (GABA) levels in the brain - Elevates the malondialdehyde (MDA) levels and reduces the antioxidant capacity. IMI could upregulate the transcription levels of nuclear factor-κb (NF-κb), interleukin-1 β (IL-1β), and tumour necrosis factor (TNF-α) genes and express strong caspase-3 and inducible nitric oxide synthase (inos) immunostaining in most examined brain areas
(Abdelhafez et al., 2023)		Adult male rats s (Rattus Norvegicus) (5/group)	0, 22, 45	Gavage, 28 days		- The effects of IMI on the central nervous system and MOA - The ache activities decreased significantly, while the concentration of malondialdehyde MDA increased significantly
(Hirai et al., 2022)		C57BL/6NCrSlc mice (8/group)	0, 10, 50	Oral administration with sonde, once		- The effect of IMI exposure on Monoamine neurotransmitters in brain - Quantification of mas in various brain regions showed a decrease in some MA levels in the olfactory bulb and the striatum - In the behavior tests, a decrease in activity was observed

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Mudgal et al., 2023)		Male albino mice (6/group)	45	Oral, 28 days		- Neuroprotective effect of ascorbic acid (AA) against neurotoxicity of IMI - Memory losses were assessed using the Y-maze and novel target identification behavioral tests - IMI-treated mice had substantial impairment of spatial and non-spatial memory functions, as well as reduced antioxidant enzyme and acetylcholinesterase activity. - IMI exposure causes oxidative stress and neurotoxicity in mice
(Saito et al., 2023)		Pregnant female C57BL/6N mice	0.06	Orally, from the gestation period (embryonic day 11.5) to maternal ablactation when pups were 4 weeks old		- Study investigated the effects of low-level, chronic neonicotinoids exposure in early life on the brain functions of adult mice impairment in learning and memory. Furthermore, immunohistochemical analysis revealed a decrease in SOX2 (as a neural stem cell marker) and GFAP (as an astrocyte marker) positive cells of the hippocampal dentate gyrus
(Tonietto et al., 2022)		Adult male Wistar rats (12/group)	0, 1.5, 5, 15	Oral gavage, 45 days		- IMI caused an increase in rearing and time spent at the periphery in the locomotor activity test and a decrease in time spent to finish the OX maze task - In blood, there was a decrease in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration and an increase in serum butyrylcholinesterase activity
(Abd-Elhakim et al., 2018)		Adult and adolescent male rats	1 mg	Oral gavage, 60 days		- The neurobehavioral performance, oxidative stress and apoptotic changes in the brain were investigated. - IMI exposure led to reduced exploratory activity, impaired sensorimotor functions, and increased depressive behavior. - Significant declines in serotonin, GABA, and dopamine levels were observed, along with oxidative brain damage marked by elevated protein carbonyl, 8-hydroxyguanosine, and malondialdehyde, while total antioxidant capacity decreased.

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration; substance	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- Brain tissue analysis showed neuronal degeneration, increased GFAP and Bax-positive cell expression, and reduced Bcl-2 expression, with more severe effects in adult rats compared to adolescents.

Table X.97: Overview on studies on eye and skin irritation and sensitisation

Reference	Cited in	Findings
Anonymous 1987 **Pauluhn 1988b**	ECHA reg dossier (FAO and WHO, 2002)	- No dermal irritation in an acute dermal irritation / corrosion test according to OECD 404 with New Zealand White rabbits (purity: 94.2%)
Anonymous 1987 **Pauluhn 1988c**	ECHA reg dossier (FAO and WHO, 2002)	- No eye irritation in an acute eye irritation / corrosion study according to OECD 405 in New Zealand White rabbits (purity: 94.2%)
Anonymous 1987 **Ohta 1988**	ECHA reg dossier (FAO and WHO, 2002)	- No sensitising potential in an in vivo skin sensitisation test according to OECD 406 with male Dunkin/Hartley guinea pigs (purity: 94.2%)

Table X.98: Overview of genotoxicity studies

Reference	Cited in	Findings
Anonymous 1987 **Herbold 1989a**	ECHA reg dossier (FAO and WHO, 2002)	- Ames test (OECD 471) negative with and without S9 up to 12,000 µg/plate, purity: 95%
Anonymous 1990 **Herbold 1991**	ECHA reg dossier (FAO and WHO, 2002)	- Ames test (OECD 471) negative with and without S9 up to 5,000 µg/plate, purity: 96%
Herbold 1991	(FAO and WHO, 2002)	- Ames test (OECD 471) negative with and without S9 up to 5,000 µg/plate, purity: 96.3%
Herbold 1992	(FAO and WHO, 2002)	- Ames test (OECD 471) negative with and without S9 up to 5,000 µg/plate, purity: 97.4%
Watanabe 1991a	(FAO and WHO, 2002)	- Ames test negative with and without S9 up to 5,000 µg/plate, purity: 93.7%
Anonymous 1989 **Herbold 1989b**	ECHA reg dossier (FAO and WHO, 2002)	- In vitro chromosome aberration study in human lymphocytes (OECD 473) induced clastogenic effects, purity: 95.2%
Anonymous 1988 **Taalman 1988**	ECHA reg dossier (FAO and WHO, 2002)	- In vitro cytogenicity / sister chromatid exchange assay in CHO cells with weak clastogenic effects, purity: 95.2%
Anonymous 2018	ECHA reg dossier	- In vitro Mammalian Cell Micronucleus Test (OECD 487) negative in human peripheral blood lymphocytes with and without S9 following 3+21 h treatment, positive following 24+24 h treatment
Anonymous 1988 **Lehn (1989a)**	ECHA reg dossier (FAO and WHO, 2002)	- In Vitro Mammalian Cell Gene Mutation Test using the HPRT and XPRT genes (OECD 476) negative in CHO cells with and without S9, purity: 95.2%
Watanabe 1990a	(FAO and WHO, 2002)	- DNA damage assay negative up to 5000 µg/plate, purity: 94.7%
Herbold 1988a	(FAO and WHO, 2002)	- Mitotic recombination assay negative up to 10,000 µg/mL, purity: 95.3%
Cifone 1988	(FAO and WHO, 2002)	- Unscheduled DNA synthesis negative up to 750 µg/mL, purity: 95.2%
Putman and Morris	(FAO and WHO, 2002)	- Sister chromatid exchange in CHO cells negative up to 1200 µg/mL, purity: 95.2%
Anonymous 2019	ECHA reg dossier	- In vivo Mammalian Erythrocyte Micronucleus Test (OECD 474) negative up to the MTD of 200 mg/kg bw/d in rats
Anonymous 1987 **Herbold 1988b**	ECHA reg dossier (FAO and WHO, 2002)	- In vivo Mammalian Erythrocyte Micronucleus Test (OECD 474) negative up to the 80 mg/kg bw/d in mice, purity: 95.3%
(Ilyushina et al., 2020)		- In vivo Mammalian Erythrocyte Micronucleus Test (OECD 474) did not induce micronucleus formation in PCE of mice bone marrow

Reference	Cited in	Findings
Herbold 1989c	(FAO and WHO, 2002)	- In vivo Mammalian Erythrocyte Micronucleus Test negative up to 2000 mg/kg bw/d in Chinese hamster bone marrow, purity: 94.6%
Anonymous 1987	ECHA reg dossier	- In vivo Mammalian Spermatogonial Chromosome Aberration Test (OECD 483) negative in mice
Anonymous 1989	ECHA reg dossier	- In vivo Mammalian Bone Marrow Chromosomal Aberration Test (OECD 475) negative up to 2000 mg/kg bw
Arslan 2015	(2020 NTP, 2020)	- In vivo Mammalian (Sprague-Dawley rats) bone marrow cells. Male rats showed susceptibility to the genotoxic effects of imidacloprid, decreased the mitotic index
(Karabay and Oguz, 2005)	(X. Zhang et al., 2023)	- Modified in vivo bone marrow chromosomal aberration test dose dependently positive but this could be due to multiple chemical exposure according to authors
Herbold 1989d	(FAO and WHO, 2002)	- In vivo sister chromatid exchange in Chinese hamster bone marrow negative up to 2000 mg/kg bw/d, purity: 95%
Völker 1990	(FAO and WHO, 2002)	- Cytogenic damage assay in mouse germ cells negative up to 80 mg/kg bw, purity: 94.1%
(Mahmoud et al., 2023)		- Study aimed to evaluate the effect of olive oil (OLO) on the genotoxicity of imidacloprid - In vivo – rats exposed to imidacloprid 22.5 mg/kg b.w. For 4, 8, 12 weeks - Malondialdehyde (MDA) and glutathione peroxidase (gpx) levels were measured in blood serum. The bone marrow from the femur was collected for the comet assay. The results revealed that exposure to IMI induced DNA damage, which was associated with increased blood MDA level and decreased gpx activity.
(Mohamed et al., 2020)		- Study aimed to evaluate the effect of olive oil (OLO) on the mutagenic effect of imidacloprid - In vivo – rats exposed to imidacloprid 22.5 mg/kg b.w. For 4, 8, 12 weeks - The results revealed that imidacloprid induced a mutagenic effect in the 8th and 12th weeks of exposure

Table X.99: Overview of carcinogenicity studies

Reference	Cited in	Species	Dosage (mg/kg bw/d)	Administration, duration	Findings
Anonymous 1990, Eiben, 1991, Eiben & Kaliner, 1991	ECHA reg dossier (FAO and WHO, 2002)	Wistar Rat (50/sex/dose)	0, 100, 300, 900, 1800 ppm (0, 5.7/7.6, 16.9/24.9, 51.3/73.0, 102.6/143.7 mg/kg bw/d for males and females, respectively)	Oral feed, 24 months, OECD 453, GLP	- Bw gain decreased from 900 ppm dose dependently - Decreased water intake in HD females - Decreased abs. And re. Organ weights in HD considered secondary to decreased bw - Total number of tumours observed and the incidence of benign and malignant neoplasms were comparable in all study groups - Most tumours were observed in the pituitary gland, thyroid, mamma and uterus, incidence independent of the dose - Liver tumours in HD males (one adenoma, one carcinoma and two cholangiocellular carcinomas) within HCD
Anonymous 1990, Watta-Gebert 1991a,b	ECHA reg dossier (FAO and WHO, 2002)	B6C3F1 mouse (50/sex/dose)	0, 100, 330, 1000, 2000 ppm (0, 20.2/30.3, 65.6/103.6, 208.2/274.4, 413.5/423.9 mg/kg bw/d for males and females, respectively)	Oral feed, 24 months, OECD 451, GLP	- No treatment related increased mortality - Slight periacinary liver cell hypertrophy and loss of vacuolation in the tubules of kidneys in HD males - Increased incidence of mineralization in the brain (thalamus) in HD animals - Greatly decreased incidences of tubular basophilia in HD males and of lymphocytosis in the renal pelvis in HD males and females might be secondary to decreased food consumption - Spectrum of neoplastic lesions in control animals was consistent with that expected in ageing mice with no evidence of a treatment-related increase in the incidence of tumour-bearing animals or of specific tumour types

Table X.100: Overview of studies regarding effects on fertility.

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Anonymous 1989 **Suter et al. 1990**	ECHA reg dossier (FAO and WHO, 2002)	Wistar rat (F0 parents: 30/sex/dose, F1 parents: 26/sex/dose), 2-Gen repro study (OECD 416, GLP)	0, 100, 250, 700 ppm (100 ppm: 5-13/6-13 mg/kg bw/d for P0 males and females during gestation, 10-18 mg/kg bw/d for P0 females during lactation, 5-9/6-9 mg/kg bw/d for F1 males and females, respectively during gestation, 11-18 mg/kg bw/ for F1 females during lactation 250 ppm: 12-32/16-32 mg/kg bw/d for P0 males and females during gestation, 25-46 mg/kg bw/d for P0 females during lactation, 12-23/16-24 mg/kg bw/d for F1 males and females, respectively during gestation, 26-42 mg/kg bw/d for F1 females during lactation 700 ppm: 35-89/44-96 mg/kg bw/d for P0 males and females during gestation, 73-121 mg/kg bw/d for P0 females during lactation, 36-68/41-68 mg/kg bw/d for F1 males and females, respectively during gestation, 78-120 mg/kg bw/d for F1 females during lactation)	Oral feed, P generation during 84-day preparing period and also during the pairing-, gestation- and lactation periods for breeding the F1A and F1B litters Purity: 94.4-95.3%	NOAEL (parental): 250 ppm, NOAEL (repro): 700 ppm	P0 - No increased mortality - Decreased bw in HD males and in HD females throughout the preparing period (sign.), gestation period, lactation period (sign.), and lactation period (sign.) - Decreased food consumption in HD males during preparing and in females during preparing (sign.), gestation (sign.), lactation (sign.) - No histopathological findings in any organ examined - Mating performance, fertility, duration of gestation, litter size normal but one HD female lost its entire f1b litter between day 6 and 12 of lactation period (considered incidental) P1 - No treatment related increased mortality - Decreased bw in HD males and in HD females throughout the preparing period (sign.), gestation period, lactation period (sign.), and lactation period (sign.) - Slightly increased food consumption in HD males, decreased in HD females during preparing (sign.), gestation (sign.), lactation (sign.) - Sign. Increased cyp and n-demethylase in HD males, slight increase o-demethylase in HD females - Sign. Decreased abs. Ovary weight in HD females but without histopathological correlations and rel. weight was similar to ctrl - No evidence of an adverse effect of treatment on mean precoital time, fertility index, conception rate and gestation index.

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- At 250 ppm sign. Increased breeding loss in f2a litters from day 4-21 postpartum was within normal range of this strain and considered incidental F1 - Bw gain decreased in HD f1a litters, in f2a litters during lactation bw gain and bw sign. Decreased - Decreased abs. Liver weights in HD f1a and f1b male and female pups, abs. Testes weight decreased in f1a pups, rel. Testes weight increased in f1b pups considered incidental since in no other generation F2 - Bw and bw gain sign. Decreased in HD f2a and f2b litters during lactation - Increased abs. And rel. Ovary weight in md f2a litters without dose relation - Decreased abs. Liver weight in HD f2b males
Mohamed et al. 2017	(2020 NTP, 2020)	Male rats (10/group - two mature and two immature groups)	1	Gavage (in corn oil), 65 days		- Study aimed to evaluate the adverse effects of imidacloprid (IMI) on male spermatogenesis, steroidogenesis, and DNA damage in sexually mature and immature rats - Both sexually mature and immature rats showed serious abnormalities in sperm morphology and concentrations, with an imbalance of sexual hormones - Increases in the level of serum 8-hydroxy-2'-deoxyguanosine and in the percentage of comet (tailed) sperm DNA in the IMI-treated groups - The upregulation of a DNA damage tolerance gene (8-oxoguanine glycosylase 1) and downregulation of the activity of steroidogenic genes (nuclear receptor subfamily 5, group A, member 1 and 3beta-hydroxysteroid dehydrogenase). Immunohistochemical examination of the B-cell lymphoma 2-associated X apoptotic protein in testicular sections showed

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						various degrees of apoptosis in the spermatogonial cells of the IMI-treated rats compared to the control groups
Bal 2012c	(2020 NTP, 2020)	Male rats	0.5-, 2, 8	Oral, 90 days		- The weights of the epididymis, vesicula seminalis, epididymal sperm concentration, body weight gain, testosterone and reduced glutathione values were lower in the imidacloprid-treated groups than that in the controls. - All treated groups had increased lipid peroxidation, fatty acid concentrations and higher rates of abnormal sperm. Apoptosis and fragmentation of seminal DNA were higher in rats treated at the two higher doses of imidacloprid
Bal 2012b	(2020 NTP, 2020)	Adult male rats	0.5, 2, 8	Oral, 3 months		- The deterioration in sperm motility in IMI-8 group and epidydimal sperm concentration in IMI-2 and IMI-8 groups and abnormality in sperm morphology in IMI-8 were significant. - The levels of testosterone (T) and GSH decreased significantly in group IMI-8 compared to the control group. Upon treatment with IMI, apoptotic index increased significantly only in germ cells of the seminiferous tubules of IMI-8 group when compared to control. - Fragmentation was striking in the seminal DNA from the IMI-8 group, but it was much less obvious in the IMI-2 one. - IMI exposure resulted in elevation of all fatty acids analyzed, but the increases were significant only in stearic, oleic, linoleic and arachidonic acids
(Abdel-Razik et al., 2021)		Male albino rat (5/group)	9	Orally by gavage, five times a week for one Month		- The testicular deficiency associated with exposure to IMI and other 2 insecticides. - Exposure resulted in a significant decline in animal body weight, sperm count, motility, normality, and viability with increased head and tail deformities - Significant reduction in serum testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), testis

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						superoxide dismutase (SOD), and reduced glutathione (GSH) levels - In contrast, catalase (CAT), lipid peroxidation (LPO), and protein carbonyl content (PCC) levels were significantly stimulated
(Keles et al., 2019)		Wistar albino male rats (6-7/group)	20	Orally (in corn oil), 8 weeks		- Study investigating the effect of vitamin D on testis morphology and irisin immunoreactivity in imidacloprid treated rats - Degenerative changes in the seminiferous tubule epithelium, interstitial oedema and increased irisin immunoreactivity - Increased TOS (total oxidant status) and decreased TAS (total antioxidant status)
(Alamgir Kobir et al., 2023)		Adult male rabbit (8/group)	Bildor® 100 mg/ L water spray on green grass)	Orally via contaminated green grass, 2 weeks		- The treatment groups received IMI contaminated green grass without wash and after wash - The thickness of tunica albuginea of testes reduced significantly with loosely arranged connective tissues in IMI-treated rabbits - Within the testes, the bizarre-shaped seminiferous tubules were seen with increased lumen diameter in IMI-treated rabbits - The spermatogenic cells were disorganized and detached from the basement membrane in seminiferous tubules of IMI-exposed testes of rabbits. - The spermatogenic cell population decreased significantly in IMI-group compared to control. - Leydig cell number decreased significantly in IMI group - A high percentage of morphologically abnormal spermatozoa was seen in IMI-treated rabbits.

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Mehanna et al., 2022)		Adult male Wistar rats (7/group)	45	Oral gavage, 60 days		- Study investigating protective effect of majorana essential oil against IMI-induced reprotoxicity - Remarkable abnormalities in both territorial aggressive and sexual behaviours were observed in IMI-treated rats with a significant elevation of serum FSH and LH as well as altered testicular redox status. Along with inhibition of the testicular expression of star and aromatase genes and serum total testosterone in addition to abnormal sperm count, viability, motility, and morphology. - Histopathological examination showed severe degeneration and necrosis in both germ cells and Leydig cells with atrophy in most of the seminiferous tubules
(Nsamou et al., 2022)		Female Rats (10/group)	22.5	Orally, 4 weeks		- Study aimed at evaluating the preventive effects of Amaranthus hybridus on the reproductive toxicity of colibri® (commercial formulation of Imidacloprid) - From day 18 of treatment till the end, half of animals in each group (n = 5) was used for the fertility test whereas the remaining rats were kept under treatment until sacrifice - Significant reduction in the ovarian and uterine weights, LH, FSH, oestradiol and progesterone levels as well as ovarian superoxide dismutase, catalase and peroxidase activities. Moreover, alteration of ovaries, uteri and vagina histology, increase in MDA concentration, decrease in fertility and parturition indices and, pup's viability were recorded
(Saber et al., 2021)		Adult male rats (10/group)	22.5	Orally, 56 days		- Study investigating the potential ameliorative effect of thymol on the testicular toxicity caused by imidacloprid - Significant decline in the body weight change, reproductive organ weights, sperm functional parameters, and serum level of testosterone, widespread histological alterations, and apoptosis in the testis. - Remarkable increment in the serum levels of follicle stimulating hormone and luteinizing hormone. Also, IMI

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						induced testicular oxidative stress, as indicated by elevated malondialdehyde (MDA) levels and a marked decline in the activity of antioxidant enzymes and reduced glutathione (GSH), and total antioxidant capacity (TAC) levels. - Moreover, IMI treatment significantly downregulated the mRNA expression of steroidogenic genes and proliferating cell nuclear antigen (PCNA) immunoexpression in the testicular tissue. H
(Sevim et al., 2023)		Wistar Albino male rats (12/group)	0, 5.7	Orally, 90 days		- The potential health-promoting effects of probiotics against intoxication by pesticides - Imidacloprid caused a degeneration and necrosis of the spermatocytes in the tubular wall, a severe edema of the intertubular region and a hyperaemia. This was concomitant to increased levels of 8-hydroxy-20-deoxyguanosine reflecting oxidative stress, and an increase in caspase 3 expression, reflecting apoptosis
(G. Zhao et al., 2021)		Male Wistar rats (10/group)	0, 0.06, 0.6	Orally, 90 days		- IMI caused abnormalities in sperm concentrations and morphologies, accompanied by an imbalance of the gonadal hormone testosterone. Histopathological damage and decrease of testosterone levels were observed in testes from rats treated with IMI. - However, oestradiol and gonadotropin levels were unchanged after IMI treatment. IMI inhibited the activity of cytochrome P450 3A4 (CYP3A4) and left itself existed in the organism of rats. - Low-dose IMI exposure caused sperm abnormalities through affecting on the spermiogenesis in testis

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
(Sood, 2023)		Female albino rats	0, 10, 20	Oral intubation, 60 days		- Disturbed cyclicity with significant decrease in the number of oestrous cycle days and increased diestrous index was observed at high dose. - Number of healthy follicles was significantly decreased with increased number of atretic follicles at high dose - Decreased body weight and ovary weight in imidacloprid treated rats was dose dependent
(Tariba Lovaković et al., 2021)		Adult male Wistar rats (5/group)	0, 0.06, 0.8, 2.25	Orally, 28 days		- Significantly lower testis weight in high dose group - Increased the level of reduced glutathione in the epididymis (73%) in low dose group, while the activities of epididymal glutathione peroxidase and superoxide dismutase significantly increased in all treated rats - Low, but significant, level of DNA damage in testicular sperm cells regardless of the concentration applied

Table X.16: Overview of studies regarding developmental toxicity

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Anonymous 1988 **Becker et al. 1988a**	ECHA reg dossier (FAO and WHO, 2002)	Wistar rats (25 dams/dose), prenatal developmental toxicity study (OECD 414, GLP)	0, 10, 30, 100	Oral gavage, GD 0-15, killed on GD21 Purity: 94.2%	Maternal LOAEL: 10 Developmental NOAEL: 30	- Maternal bw gain decreased at MD, sign. At HD - Maternal food consumption decreased in all treatment groups, increased in HD after end of treatment - In foetuses a change in sex ratio was observed (m/f 58.6/41.4% compared to 51.0/49.0% in ctrl) but this was not seen in the dose range finder study at 150 mg/kg bw/d and was shifted at 100 mg/kg bw/d in favour of females - Wavy ribs more frequently in HD foetuses - Visceral malformations in form of severe haemorrhage around the brain and dilated lateral ventricles of the brain - Enlarged heart (auricles were dilated with blood and the walls of the ventricles were thickened) - Considered secondary to maternal toxicity
Anonymous 1988 **Becker et al. 1988b**	ECHA reg dossier (FAO and WHO, 2002)	Chinchilla rabbits (16 dams/dose), prenatal developmental toxicity study (OECD 414, GLP)	0, 8, 24, 72	Oral gavage: GD 6-18, killed on GD 28 Purity: 94.2%	Maternal NOAEL: 8 Developmental NOAEL: 24	- Abortion in one HD female - 2 HD dams died - Bw loss and decreased food consumption in MD and HD - Increased post implantation loss in HD - Total litter resorption in two HD females - Fatus bw sign decreased in HD - Live HD foetuses decreased due to abortion and post implantation loss - Foetal HD bw decreased - Increased number of non-ossified phalangeal nuclei and metacarpalia of both fore limbs and phalangeal nuclei of both hind limbs considered secondary to maternal tox

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Bhaskar 2017	(2020 NTP, 2020; Ongono et al., 2020b)	Swiss albino mice (12 pregnant mice/group; 15-17 F1 mice/group)	0, 0.65	Through diet, PND 1-28		- Working memory using the T-maze test on PND 29 and 63 - Mice were exposed through the diet of lactating mothers from post-natal day (PND) 1 to PND 28. - Results were reported only in male pups. Imidacloprid did not alter working memory performances in the offspring
Bhaskar and Mohanty 2014	(NTP, 2020)	Mice	0, 0.65	Oral diet,		- Study investigating if coexposure of dithiocarbamate fungicide mancozeb (MCZ) and imidacloprid (IMI) during lactation increased the risk of body weight gain in mice later in life. - Body weight gain has been linked to pesticide-induced hypothyroidism and hyperprolactinemia and alteration of lipid profiles. - In vivo results were substantiated with in silico molecular docking (MD) analysis that predicted the binding affinity of pesticides with thyroid hormone receptors (trα and trβ) and peroxisome proliferator activated receptor gamma (pparγ), the major nuclear receptors of peripheral fat metabolism. Binding potency of MCZ and IMI was compared with that of T3, and its antagonist ethylene thiourea (ETU) as well as pparγ agonist (rosiglitazone) and antagonist (HL005). - Individual pesticide exposure in low dose might not exert the threshold response to affect the receptors signalling further to cause hormonal/metabolic impairment.
Abou-Donia et al. 2008	(EFSA Panel on Plant Protection Products and their Residues (PPR), 2013; 2020 NTP, 2020)	Pregnant Sprague-Dawley rats	0, 337	Intraperitoneal injection, GD9		- On postnatal day (PND) 30, all male and female offspring were evaluated for (a) acetylcholinesterase (ache) and butyrylcholinesterase (buche) activity, (b) ligand binding for nicotinic acetylcholine receptors (nachr) and muscarinic acetylcholine receptors (m2 machr), (c) sensorimotor performance (inclined plane, beam-walking, and forepaw grip), and (d) pathological alterations in the brain (using cresyl violet and glial fibrillary acidic protein [GFAP] immunostaining).

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
						- The offspring of treated dams exhibited significant sensorimotor impairments at PND 30 during behavioral assessments. - Increased ache activity in the midbrain, cortex and brainstem (125-145% increase) and in plasma (125% increase). - Ligand binding densities for [3H]cytosine for alpha4beta2 type nachr did not show any significant change, whereas [3H]AFDX 384, a ligand for m2machr, was significantly increased in the cortex of offspring (120-155% increase) of imidacloprid-treated mothers. - Histopathological evaluation using cresyl violet staining did not show any alteration in surviving neurons in various brain regions. On the other hand, there was a rise in GFAP immunostaining in motor cortex layer III, CA1, CA3, and the dentate gyrus subfield of the hippocampus of offspring of imidacloprid-treated mothers.
Gawade et al. 2013	(NTP, 2020)	Female Wistar rat	0, 10, 30, 90	Oral gavage, GD 6- GD 21, GD6-PND21, PND42		- Immunotoxicity parameters - Post-implantation loss in the highest dose group, indicating the risk of abortion. - Soft tissue abnormalities and skeletal alterations were observed in the highest dose group. - Humoral immunity was assessed by estimating hemagglutination titer and immunoglobulin production. - Imidacloprid caused age-dependent adverse effects on the developing immunity which was aggravated when exposure continued throughout development, leading to a compromised immune system.
(Haddad et al., 2023a)		Wistar albino rats (1/group)	0, 1.2	Oral, from day 1 of gestation to day 21 of lactation, adult females of the F1 generation were mated with males (not		- Study investigating the effect of imidacloprid and cypermethrin either alone or in a mixture on behavior and mitochondrial redox status in two generations of pups. - Induced decreased body weight gain, impaired neurobehavioral performance, and ache inhibition - Oxidative stress markers including GSH level and SOD, CAT, GST and gpx activities showed a significant decrease.

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
				exposed to pesticides) to Generate the F2 generation		- While lipid peroxidation which was assessed by MDA assay, cytosolic calcium level, swelling and mitochondrial permeability recorded a significant increase - The group size is not explicitly mentioned, but according to the study "After fertilization, 4 females (F0) were placed individually in cages. They were then divided into 4 exposure groups", which would lead to a group size of 1 and is therefore unsuitable for evaluation
(Ndonwi et al., 2020)		Female nulliparous Wistar rats (6/group)	0, 44	Orally, throughout gestation		- Offspring were explored at weaning (4 weeks) and adult age (12 weeks) - Significantly higher fasting glucose and insulin levels - IMI induces hyperglycaemia, insulin resistance and dyslipidemia
(Sardar et al., 2023)		Neonatal male rats (10/group)	0, 1, 5, 10	Orally, PND1-PND26		- The results of body weight gain showed a significant difference in group 4 (10 mg/kg) animals as compared to the control - A significant increase in total cholesterol and triglycerides, while a decrease in high-density lipoprotein concentrations - Significant decrease in concentrations of antioxidant enzymes (CAT and SOD) among all the IMD-treated groups - Increased production of ROS was also noticed in the highest-dose treatment group - Histopathological changes in the testis and epididymis along with a significant decrease in the plasma testosterone concentrations among IMI-treated groups - An increase in lumen diameter and a decrease in epithelial height of seminiferous tubules were also observed

Table X.101: Overview of studies with effects on immunotoxicity

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
Anonymous 2010	ECHA reg dossier	Wistar rats (10 males/dose)	0, 150, 600 or 2400 ppm (0, 11.7, 47.1, 186 mg/kg bw/d)	Dietary, 28 days	NOAEL for immunotox: 186,	- No increased mortality - Piloerection and wasted appearance in one HD male

Reference	Cited in	Species, study type	Dosage (mg/kg bw/d)	Administration, duration	NOAEL / LOAEL (mg/kg bw/d)	Findings
		Short-term immunotoxicity (EPA OPPTS 870.7800, GLP)			NOAEL for general tox: 47.1	- Bw, bw gain and food consumption decreased in HD - No immunological findings - Abs. thymus weight and rel. spleen weight decreased, considered secondary to decreased bw and within HCD - Atrophic/small thymus in HD (3/10) and congested/red intestine (3/10) were considered secondary to general toxicity

Part XIa

Human biomonitoring guidance values (HBM-GVs) for **elemental and inorganic mercury** for workers and the general population

Technical reference

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Lead Beneficiary/ Responsible AE	TTL
Contributing Participants	Helle Katrine Knutsen, NIPH, hellekatrine.knutsen@fhi.no Petra Apel, UBA, petra.apel@uba.de, Lucija Perharič (NIJZ), lucija.perharic@nijz.si
Responsible author(s)	Jonna Weisell-Laitinen, Finnish Institute of Occupational Health, jonna.weisell-laitinen@ttl.fi
Co-authors	Tiina Santonen, Finnish Institute of Occupational Health, tiina.sanotonen@ttl.fi Helle Katrine Knutsen, NIPH, hellekatrine.knutsen@fhi.no Petra Apel, UBA, petra.apel@uba.de
Internal Reviewers[2]	WP4 co-leaders
External Reviewers[3]	Experts from Finnish NH, Greek NH, Polish NH, Spanish NH, Swiss NH, UK NH Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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[2] WP/task co-leader(s) from the same WP

[3] Independent Reviewers that should not be part of the task related to the AD/D or part of projects related to the Task

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Version 2	25.01.2025	Jonna Weisell-Laitinen	Draft version considering comments provided by partners
	14.02. - 10.03.2025	Experts from Finnish NH, Greek NH, Irish NH Polish NH, Spanish NH, Swiss NH, UK NH Partner: Lucija Perharič (NIJZ)	See Annex to AD 4.3
Version 3	11.03.2025	For approval by P4.1.3.1.a partners	Draft version considering comments provided by NH experts
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Abstract

Human biomonitoring guidance values (HBM-GVs) help interpret data from human biomonitoring in relation to health. This allows biomonitoring to be used in evaluating and managing chemical risks to humans. As part of the PARC (Partnership for the Assessment of Risks from Chemicals) project, HBM-GVs are established for chemicals that are significant in environmental and occupational settings, following a strategy developed in the previous EU project, HBM4EU.

In this document, HBM-GVs are proposed for elemental and inorganic mercury for workers and the general population. During the HBM4EU project a provisional HBM-GV for inorganic mercury including elemental mercury for the general population was proposed. This value was provisional as it did not go through the whole consultation round that was agreed in the project. In addition, no HBM-GV was derived for the workers.

Workers are mainly exposed to mercury vapor via inhalation. The general population is more prominently exposed to mercury vapor through dental amalgams than to inorganic mercury through the diet. Neurotoxicity is considered as the most sensitive endpoint for elemental and inorganic mercury. Both proposed HBM-GVs for the general population and workers are based on occupational studies on elemental mercury suggesting effects at calculated cumulative urinary exposures of >12.4 µg Hg/g creatinine/year. This value, representing a biological value causing no effect in the studied endpoint is chosen as a point of departure.

Since the exposure data is based on several occupational studies pointing in the same direction and including in total hundreds of workers, inter-individual variability among workers is considered to be sufficiently covered by the data and no additional assessment factors are needed. This results in an HBM-GV of 12 µg Hg/g creatinine or 17 µg Hg/l in urine for workers.

For the general population, an additional assessment factor 2 is used to cover vulnerable groups (such as children and the elderly) and to take into account lifetime exposure. This results in a value of 6 µg Hg/g creatinine or 7.5 µg Hg/l in urine for the general population. The proposed HBM-GV is almost the same as the earlier provisional general population HBM-GV of 7 µg Hg/l for inorganic mercury previously derived within the HBM4EU project. This was based on animal data showing kidney effects after oral exposure to inorganic mercury, and takes into account also e.g. developmental toxic effects observed in animal tests at higher doses. As the urine mercury concentration does not differentiate between elemental mercury and inorganic mercury, the HBM-GV should cover both elemental and inorganic mercury. The provisional HBM-GV of 7 ug/l derived within HBM4EU for inorganic mercury can be considered to be protective also from the effects observed in mercury vapor exposed humans. Therefore, an HBM-GV of 6 µg Hg/g creatinine (7 µg Hg/l) is proposed and will replace the earlier provisional value derived within the HBM4EU project.

Key Words

HBM-GV, human biomonitoring, human biomonitoring guidance values, elemental mercury, mercury vapor, inorganic mercury, metals, general population, workers

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Authors and Acknowledgements

Authors:

Jonna Weisell-Laitinen, Tiina Santonen, Helle Katrine Knutsen, and Petra Apel

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Results

In this document, HBM-GVs are proposed for elemental and inorganic mercury for workers and the general population. For workers an HBM-GV of 12 µg Hg/g creatinine or 17 µg Hg/l in urine was derived and justified. For the general population an additional assessment factor 2 was used to cover vulnerable groups (such as children and the elderly) and to take into account lifetime exposure. Therefore, an HBM-GV of 6 µg Hg/g creatinine (7 µg Hg/l) is proposed replacing the previous provisional value derived in the HBM4EU project at the same level. As the mercury concentration in urine does not allow a distinction to be made between elemental mercury and inorganic mercury, the HBM-GV includes both elemental and inorganic mercury.

Abbreviations and Acronyms

ADHD	Attention-deficit/hyperactivity disorder
AF	Assessment Factor - Assessment factors are used to address the differences between experimental data and the human situation, taking into account the uncertainties in the extrapolation procedure and in the available data set.
ATSDR	Agency for Toxic Substances and Disease Registry
BAT	Biological tolerance value for occupational exposure
BLV	Biological limit value
BMD	Benchmark Dose Modelling
BMDL	Benchmark dose lower bound
CONTAM	The Panel on Contaminants in the Food Chain
EFSA	European Food Safety Authority
EU	European Union
GLP	Good Laboratory Practice
HBM4EU	Human Biomonitoring for EU
HBM-GV	Human Biomonitoring Guidance Value
HBM-I	The concentration of a substance in human biological material at and below which there is no risk for adverse health effects
HBM-II	The concentration of a substance in a human biological material at and above which there is an increased risk for adverse health effects
IAP	Intestine alkaline phosphatase
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JEM	Job-exposure matrix
ILO	International Labour Organization
NAG	N-acetyl-beta-glucosaminidase
NOAEL	Non-Observed Adverse Effect Level
LOAEL	Lowest observed adverse effect Level
PBPK	Physiologically based pharmacokinetic modelling
POD	Point of Departure
PTWI	Provisional tolerable weekly intake
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCOEL	The Scientific Committee on Occupational Exposure Limits
TNAP	Tissue non-specific alkaline phosphatase
TWI	Tolerable Weekly Intake
UBA	German Environment Agency
WHO	World Health Organisation

1 Introduction

Under PARC, WP4, project 4.1.3.1.a, Human Biomonitoring Guidance Values (HBM-GVs) are derived for priority substances. These HBM-GVs can be directly compared with HBM data and, as jointly agreed values under PARC, they are a valuable tool for the harmonized assessment of health risks. The HBM-GVs are derived as needed for the general population and/or occupationally exposed adults.

The human biomonitoring guidance values for the general population (HBM-GV_{GenPop}) represents the concentration of the substance (or its metabolites) in human biological material up to which, according to current knowledge, there is no risk of adverse health effects from that individual substance. HBM-GVs are mostly set for substances and metabolites measured in urine, but rarely blood or other tissues. They generally do not consider combined effects of different substances. An exceedance of this value should be a reason to investigate the causes and to minimize possible sources of exposure.

The HBM-GVs can be derived also for occupational exposure. HBM-GVs derived for occupationally exposed adults (HBM-GV_{Worker}) represent a concentration of a substance or its relevant metabolite(s) in human biological media aiming to protect workers exposed to the respective substance from the adverse effects of the regular exposure over the course of the working life (Apel et al., 2020).

Under the European HBM4EU project, derivation of HBM-GVs was started. For mercury, provisional HBM-GVs for the general population were proposed for inorganic mercury including elemental mercury (Apel et al., 2022). However, HBM-GVs for workers were not derived within the HBM4EU project. Therefore, an HBM-GV for workers was derived within PARC. The provisional HBM-GV for inorganic mercury for the general population was updated. Additionally, literature search on elemental mercury exposure in the general population was prepared within the HBM4EU project but no HBM-GV was proposed based on this data.

In this report, HBM-GV for elemental mercury for the general population is also derived. Toxicological evaluations are based on previous evaluation conducted during the HBM4EU project and on the recent Agency for Toxic Substances and Disease Registry (ATSDR) report on mercury (Apel et al., 2022; ATSDR, 2024). This document will be an amendment to the HBM4EU deliverable for mercury, and therefore it includes only the key studies for the derivation of the HBM-GV_{worker} as the more comprehensive description of toxicology data can be found in the earlier HBM4EU deliverable (Apel et al., 2022) and ATSDR (2024) report of mercury. HBM4U deliverable describes new studies for inorganic mercury following EFSA (2012) report and for elemental mercury deliverable reported main aspects of previous report and recent reviews. Selected key studies are identified as a relevant study in the previous evaluations of inorganic mercury or elemental mercury made by other authorities.

2 HBM-GV for Workers

2.1 Background

2.1.1 Exposure

The use of and exposure to mercury has been described in the deliverable 5.12 of the HBM4EU project. The key information on occupational exposure is described below. In the EU, regulation 2017/852 and 2024/1849 restricts the use of mercury. The export of elemental mercury and seven mercury compounds is prohibited, with the only exception being for analytical purposes. In addition, the export of mercury compounds for the purpose of reclaiming mercury is prohibited, including the exportation of mercury compounds and of mixtures of mercury. The import of elemental mercury and seven mercury compounds as well as mercury mixtures is prohibited. The exception to this is related to waste disposal when the exporting country has no access to mercury conversion to a non-toxic form within its own territory and when the mercury source is not from mining. In addition, export, import, and manufacturing in the European Union of the mercury-added products is prohibited with exception of products that are essential for civil protection and military uses, products for research, for calibration of instrumentation, or for use as a reference standard.

In addition, there are restrictions on the use and storage of mercury, mercury compounds and mixtures of mercury. More precisely, prohibited manufacturing processes are those where mercury or mercury compounds are used as a catalyst, in the production of polyurethane, and in manufacturing processes in which mercury is used as an electrode. The use and release of mercury, mercury compounds or mercury mixtures in the production of sodium or potassium methylate is restricted and will be prohibited from 1 January 2028. Artisanal and small-scale gold mining and processing in which mercury amalgamation is used to extract gold from ore is prohibited.

From the beginning of 2025, the manufacture, use and export of dental amalgam is prohibited. Certain mercury containing lamps for general lightning have been banned from the beginning of 2019. In addition, the manufacture and export of six additional mercury containing lamps will be prohibited from 1 January 2026 and 1 January 2028 (depending on the lamp type).

Workers have traditionally been occupationally exposed to elemental mercury and inorganic mercury. Due to the Minamata convention and EU legislation occupational exposure to mercury has been decreasing. However, there are still relevant sources of occupational mercury exposure. In 2019, the following mercury containing products were used in the EU: batteries, accumulators, electrical and electronic devices, non-electrical or electronic devices, lamps, medicinal products, dental amalgams, cosmetic products, paints and varnishes, biocides, and other mercury added products. Workers have been exposed to elemental and/or inorganic mercury in the chloralkali industry, production of batteries, vinyl chloride monomer and pigment production, and handling of following products in all stages of product life cycle: dental amalgams, fluorescent and mercury discharge lamps, pressure measuring instruments, (ILO, 2022). Due to these uses, occupational exposure to mercury may occur in the waste and recycling industry.

Inhalation is the main exposure route in workers, but exposure can be also through skin absorption or ingestion. Elemental mercury easily evaporates resulting in inhalation exposure.

The general population is mainly exposed to organic mercury orally through food, especially through fish consumption (Apel et al., 2022; ATSDR, 2024). Another major exposure source is elemental mercury due to the use of dental amalgams as they continuously release mercury vapor even after placing the dental amalgams (ATSDR, 2024; Richardson et al., 2011). Studies show increasing urine mercury concentration with increasing number of dental amalgams (ATSDR, 2024; Richardson et al., 2011). In children, urine mercury concentration declined after the replacement of dental amalgams with resin restorations (ATSDR, 2024). It has been estimated that dental amalgams may result in the intake of mercury ranging from 3.8 to 21 µg/day (Apel et al., 2022; WHO, 1990). Richardson et al. (2011) estimated that average exposure for children is 0.04 µg Hg/kg bw/day and for adults 0.07 µg Hg/kg bw/day. The general population can be also exposed to inorganic mercury, but it is generally minor exposure when compared to organic and elemental mercury (ATSDR, 2024). However, this may change due to the ban of dental amalgams starting from 2025. Sources of inorganic mercury may include contaminated soil, drinking water or non-fish food. The mean exposure of inorganic mercury through diet has ranged in Europe between 0.26-

0. 53 µg/kg body weight per week and mean 95th percentile have ranged 0.53-1.66 µg/kg body weight per week in adults (EFSA, 2012). Urinary mercury 95th percentile levels in general population (adults) has ranged from 1 µg/l (Germany) up to 6.6 µg/l (Northern France) (Apel et al., 2022).

2.1.2 Toxicokinetics

Elemental mercury, Hg^0, is a volatile compound; thus, the main exposure route is inhalation. Elemental mercury is readily absorbed through the lungs in humans with an retention rate of approximately 69-85% (ATSDR, 2024). Elemental mercury is not well absorbed through the skin but due to its volatility, inhalation exposure also is possible when exposed dermally. Dermal absorption is estimated to be 2.6 % during full-body immersion in mercury vapor (ATSDR, 2024). Ingested mercury vapor is poorly absorbed from the gastrointestinal tract (ATSDR, 2024). Mercury vapor that can be inhaled is emitted by dental amalgams. Also, if the amalgam is abraded, amalgam particles can enter into the gastrointestinal tract and be oxidized (ATSDR, 2024). However, absorption is poor as it has been estimated to be 0.04 % in adults (ATSDR, 2024).

Mercury vapor can be transported via erythrocytes or hemoglobin in the blood (Berlin et al., 2015). Due to its high lipid solubility, mercury vapor is distributed rapidly into the organs throughout the body. It can also cross the blood-brain-barrier and the placenta. Mercury vapor, Hg^0, is rapidly oxidized to mercuric mercury, Hg^{2+}, in the blood and tissues and may saturate oxidation pathway (ATSDR, 2024). Saturation is due to the availability of intracellular hydrogen peroxide for catalase enzyme which is the enzyme catalyzing the main oxidative pathway (ATSDR, 2024). Oxidation of Hg^0 is a limiting step for the distribution of Hg^0 into the tissues from the blood by removing Hg^0 from the blood. Hg^{2+} is more water soluble than Hg^0. Hg^{2+} binds tightly into sulfhydryl groups in proteins. From the blood, Hg^{2+} may be distributed in the kidneys and liver (ATSDR, 2024). Despite of the rapid oxidation mercury vapor can be transported into the brain before oxidization due to rapid vascular perfusion of the brain, (Berlin et al., 2015). Oxidized mercury accumulates into the CNS due to its positive charge and inability cross back through blood-brain-barrier. Excretion of mercury vapor may be through exhaled air in unmetabolized Hg^0 or through kidney and feces in oxidized Hg^{2+} (ATSDR, 2024). Shortly after exposure, excretion of Hg^0 to exhaled air is the primary elimination route being 10 % of inhaled dose, whereas 2% is excreted into the urine and 9% into the feces. However, excretion in the urine increases with time after exposure, and excretion in the urine has been estimated to be 55 % following one year of exposure, with exhaled air elimination being approximately 14% after this duration. The terminal half-life of the mercury vapor is between 30-90 days and it reflects urinary and fecal excretion of Hg^{2+} (ATSDR, 2024).

Mercuric oxide has been shown to be absorbed following inhalation exposure via accidental exposure (ATSDR, 2024). It is distributed to the lungs, the head, the kidneys, the pelvis, and the leg regions. Orally, 2-38% of inorganic mercury (Hg^+ and Hg^{2+}) has been shown to be absorbed in laboratory animals following exposure (EFSA, 2012). In humans, absorption of inorganic mercuric mercury (Hg^{2+}) has been estimated to be 1-16% (ATSDR, 2024). Inorganic mercurous compounds (Hg^+) such as calomel are less water soluble, thus, likely less absorbed than inorganic mercuric compounds (Hg^{2+}) such as mercuric chloride (ATSDR, 2024). However, toxicokinetic data from Hg^+ is limited. The Hg^{2+} compound, mercuric chloride can be absorbed through the skin (ATSDR, 2024). In the blood, inorganic mercury is distributed in the plasma and to some extent in the erythrocytes. In plasma Hg^{2+} is bound to sulfhydryl groups in proteins. Inorganic mercury is mainly accumulated in the kidneys, followed by the liver but also into the brain, the intestinal mucosa, the testes, and the skin (ATSDR, 2024; EFSA, 2012). Inorganic mercury compounds do not readily pass blood-brain-barrier or placenta due to low lipophilicity (EFSA, 2012). Mechanism of placental transfer still needs to be characterized (ATSDR, 2024). Hg^{2+} originated from inorganic mercury is also bound to into sulfhydryl groups in proteins (ATSDR, 2024). Hg^+ is oxidated to Hg^{2+} in the blood. Hg^{2+} can be reduced to Hg^0 as exhaled Hg^0 has been detected following parenteral dose of mercuric chloride (ATSDR, 2024). Inorganic mercury is largely excreted in the urine, but also into the feces. Both elemental and inorganic mercury has some elimination through salivary and sweat glands. The half-life of inorganic mercuric mercury is approximately 40 days (EFSA, 2012).

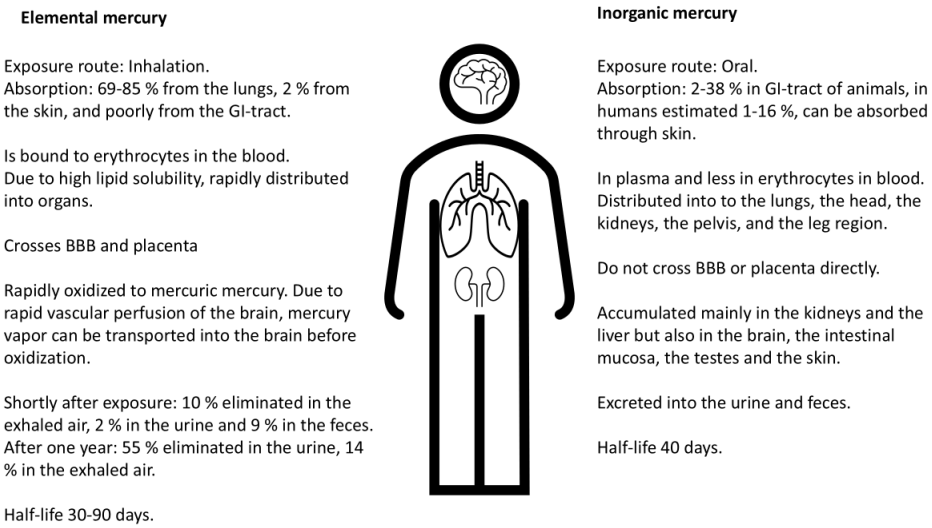

Figure XIa.11: Summary of the toxicokinetic of elemental and inorganic mercury

2.1.3 Toxicology profile of elemental and inorganic mercury

Toxicological effects have been extensively described in the HBM4EU deliverable especially for the general population (Apel et al., 2022). In the more recent ATSDR report, occupational toxicology data is also discussed in detail. In this report, only key studies for the selection of the point of departure (POD) will be described in detail. Elemental mercury has been shown to cause neurotoxic and kidney toxic effects in humans and animals (ATSDR, 2024). Neurological effects in humans include tremors, impairment of color vision and visual memory, impairment in nerve conduction velocity, motor speed, and cognitive performance as well as psychological symptoms. In animals, some evidence of neurodevelopmental toxicity has been detected. Kidney effects in humans include glomerular impairment and tubular injury. These effects are supported by animal data, which shows dose- and time-dependent nephrotoxicity. Following acute inhalation exposure, respiratory toxicity (lung inflammation, pneumonitis, pulmonary oedema, and respiratory failure) has also been described (ATSDR, 2024).

Specifically for inorganic mercury, ATSDR (2024) did not find epidemiological data. This is because the exposure of the general population to inorganic mercury compounds is limited. In workers the major exposure source is also elemental mercury and mercury vapor. In animal studies, inorganic mercury has been shown to cause mainly neuro- and kidney toxicity but there is also some evidence on cardiotoxicity, immunotoxicity and reproductive toxicity. In addition, some evidence of the carcinogenic effects of inorganic mercury were found in male rats.

Overall, carcinogenicity data in animals has been considered equivocal (EFSA, 2012; SCOEL, 2007). IARC has stated that elemental and in organic mercury are not classifiable for their carcinogenicity to humans (Group 3). Neurological effects of inorganic mercury in animals include neurodevelopmental toxicity (hyperactivity, impairment of motor coordination, memory and learning, and reduced sociability), and in adult animals neurobehavioral effects (hyperactivity, impairment of motor coordination, memory and learning), hindlimb crossing, ataxia, tremor, and partial paralysis, as well as neuropathological changes in sensorimotor regions of the central nervous system (ATSDR, 2024).

Nephrotoxicity has been dose- and time related (ATSDR, 2024). Nephrotoxic effects have been found in proximal tubules, distal tubules, and glomerular membrane. In addition, loss of brush border membranes and necrosis have been detected. In acute poisonings in humans, inorganic mercury salts have shown toxicity in the proximal tubule, confirming the human relevance of animal studies (ATSDR, 2024). There are only a few general population studies specific for inorganic mercury or elemental mercury which have studied the relationship of urine mercury concentration and renal effects. An EFSA report and the HBM4EU deliverable concluded epidemiological studies to

be insufficient to use for POD but they do confirm the relevance of animal studies in humans (Apel et al., 2022; EFSA, 2012).

In children, there are two studies that were not included in the ATSDR report, suggesting neurotoxicity at urinary mercury levels of 0.3-1.0 µg/l (Al-Saleh et al., 2020; Pan et al., 2018). At these low levels association with decreased IQ score has been also suggested. However, studies have shown inconsistent results, and cannot to be used to set a HBM-GV (Apel et al., 2022)).

In the HBM4EU report it was stated that there are little data on the reproductive effects of elemental mercury (Apel et al., 2022). SCOEL (2007) has concluded that available information on reproductive and developmental toxicity of elemental mercury is insufficient to determine NOAEL (no observed adverse effect level) and it is expected that NOAEL value would be higher than for kidney and central nervous effects. In animals, inorganic mercury has caused developmental toxicity (fetal resorptions and abnormalities) and reproductive toxicity (oestrus cycle and ovulation changes) following oral exposure (EFSA, 2012). In the EFSA report three multigenerational animal studies were identified, of which one showed effects at a low dose (0.022-0.29 mg/kg bw/d). However, this study had limitations (only one dose, unusual high survival rate for 3 years in all 3 generations of untreated control group).

Thus, " the *CONTAM Panel did not consider the data sufficiently robust to be used as a basis for establishing a health-based guidance value"* (EFSA, 2012). For other two studies CONTAM panel noted that "*Although NOAELs were not established in these two studies, the lowest reported levels for reproductive effects are three times higher than the lowest $BMDL_{10}$ for kidney effects of 0.06 mg/kg/bw/d (expressed as mercury) used as the reference point for establishing the JEFCA PTWI*" (EFSA, 2012). EFSA concluded the kidney to be critical target of inorganic mercury with BMDL10 of 0.06 mg/kg Hg/day (EFSA, 2012). Similarly, SCOEL concluded that reproductive and developmental toxicity occurred only at high exposure levels that cause maternal toxicity. ATSDR (2024) concluded that it was not possible to determine whether there are associations between elemental mercury exposure and adverse reproductive outcomes in males and females. There are only few studies that have been conducted and most of them have not shown any effects. In case of inorganic mercury, there is no epidemiological data available on the association between exposure to elemental mercury and reproductive effects. However, animal studies indicate dose-related impairment in fertility in male and female rodents following oral exposure. As described earlier, these occur however only at significantly higher levels than renal effects.

In the opinion of Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) effects of dental amalgams on developmental neurotoxicity was evaluated (SCENIHR, 2015). SCENIHR concluded that there is no evidence that amalgam negatively influences the neuropsychological development of children. After the SCENIHR report few studies have been published. Lygre et al. (2018) did not find significant associations between Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms and prenatal exposure to amalgam fillings in children of 3- and 5-years in a large prospective cohort. Lin et al. (2018) studied the association between dental amalgam restoration and risk of ADHD in children (mean age 9.6-years) in a nationwide longitudinal study (n=88 068). It was found that 6 or more amalgam restorations showed a higher risk of future ADHD in the unadjusted Cox proportional hazard regression model.

However, after adjustment for age no association was detected. Lee et al. (2018) studied association between ADHD and urine mercury concentration in children with inattentive type (n=29) or hyperactivity/impulsive type (n=47) of ADHD and in healthy controls (n=46). Urine mercury was positively correlated to hyperactivity/impulsivity scores rated by parents. However, study did not adjust for other confounding factors such as lead although they also found an association between lead exposure and both subtypes of ADHD. A recent systematic literature review evaluated an association between mercury exposure and ADHD (Farmani et al., 2024). No significant association with any ADHD subtype and mercury levels in teeth were detected (Farmani et al., 2024).

Overall, it can be concluded that the available developmental and reproductive toxicity data is not appropriate for HBM-GV derivation for elemental and inorganic mercury.

Based on the previous evaluations (ATSDR (2024), DFG (2012; 2016), UBA (1999), SCOEL (2007), EFSA (2012), and HBM4EU (2022)), the key studies for the setting of HBM-GV for elemental and inorganic mercury were identified. These studies are listed in the Table XIa.104. These studies include human data in workers exposed to mercury (mainly mercury vapor) focusing on neurotoxicity and kidney toxicity. These identified neurotoxicity and

kidney toxicity studies were included in this deliverable. In addition, two animal studies on inorganic mercury are described.

2.1.4 Neurotoxicity studies

Key studies

Bast-Pettersen et al. (2005) studied neurological effects in chloralkali workers previously exposed to mercury vapor. The study included 49 previously exposed male workers and 49 age- and sex-matched controls. The cumulative mean urinary concentration was calculated to be 9.3 nmol Hg/mmol creatinine/year (range 4.0–25.4) corresponding to 16.5 µg Hg/g creatinine/year (range 7.1–45) and the average of exposure time was 13.1 years. The cumulative urinary concentration was based on historical urinary measurements. Each participants cumulative exposure was calculated summing up mean yearly urine mercury concentrations and divided by the exposure time. Mean year urine mercury concentration was calculated from everyone's mean urine mercury concentration for each quarter of the year of exposure.

Calculated cumulative urinary concentration gives information of the exposure during the whole exposure period. The time from the previous exposure was on average 4.8 years (range 4.2-10.0). Mean urine mercury concentration at the time of the examination was for workers 1.65 nmol/mmol creatinine (range 0.2-5.2) and for controls 1.15 nmol/mmol creatinine (range 0.3-3.2). There were no differences between the workers and controls in neurobehavioral test scores and the number of subjective symptoms. 41 of the workers and 40 of controls were studied also when workers were still exposed to mercury. Neurobehavioral tests were similar in both groups. In exposed workers, the digit symbol test (analysis of psychomotor speed, attention, perceptual organization, and visual short-term memory) results showed a negative association with increasing blood mercury concentrations. An improvement in the test results was observed after the cessation of the exposure. Thus, no permanent effects of mercury vapor exposure were observed at these low exposure levels.

Ellingsen et al. (2001) studied neurological effects in chloralkali workers to mercury vapor. The study included 47 previously exposed male workers and 47 age-and sex-matched controls. Cumulative urinary concentration was based on historical urinary measurements. Cumulative mean urinary concentrations were calculated to be 9.0 nmol Hg/mmol creatinine/year (range 4.0-19.6) corresponding to 16.0 µg Hg/g creatinine/year (range 7.1–35) and the average exposure time was 13.3 years. Current mean urine mercury concentrations were 5.9 nmol Hg/mmol creatinine (range 1.1-16.8) corresponding to 10.5 (2.0–30) µg Hg/g creatine for workers and 1.3nmol/mmol creatinine (0.2-5.0) for controls. No differences in the neurological effects, including tremor, between the groups were detected between the groups. Only exception is the association with the result of digital symbol test and cumulative urine mercury concentration. There were no association with the current urine mercury concentration and the results of digital test results. Authors concluded that suggested effects were small and probably insignificant.

Fawer et al. (1983) studied hand tremors in male workers exposed to mercury vapor. Exposed (n=26) and non-exposed controls (n=25) were from chloralkali plant, fluorescent tube factories, and chemical industry. Non-exposed group were not occupationally exposed to mercury. Average exposure time was 15.3 (SE±2.6) years. The mean urinary concentration in exposed workers was 20.1 (SE 2.1) µg Hg/g creatinine and in non-exposed 6.0 (SE 1.2) µg Hg/g creatinine. Tremor was studied by holding the hand in the same position with and without the load. Tremor was significantly higher in the exposed workers than non-exposed.

Langworth et al. (1992a) studied neurological effects in chloralkali workers (n=89) versus control group (n=75). The average exposure time was 13.5 (±8.7 SD) years and the median urinary concentration in exposed workers was 25.4 µg Hg/g creatinine (range 0.5-83.3). The median urinary concentration in controls was 1.9 µg Hg/g creatinine (range 0-7.6). There were no significant changes in tremor test or psychometric tests. Tremor results were from 85 workers and 69 controls. However, self-reported symptoms were higher in the exposed group than in the control group. These self-reported tests included the scores for tiredness and confusion in the profile of mood states (POMS), and the degree of neuroticism in the Eysenck personality inventory. Also, a weak dose-response was detected between symptoms prevalence and current urinary and blood mercury concentrations and a negative correlation was found between performance of three psychometric tests and earlier peak exposures. As a summary authors indicated a slight mercury induced effect on CNS in exposed workers.

Wastensson et al. (2006, 2008) studied tremors in chloralkali male workers (n=43) and age- and sex-matched unexposed referents (n=22). The median mercury urinary concentration in exposed workers was 5.9 µg Hg/g creatine (range 1.3-25) and in referents was 0.7 µg Hg/g creatine (range 10.2-4.1). The average exposure time was 15 years and the median exposure time was 13 years. The mean cumulative index was 266 years ×µg/g creatinine and the median cumulative index was 161 years ×µg/g creatinine (range 8–1,440 years ×µg/g creatinine). The cumulative exposure index was calculated based on previous biomonitoring data. If exposure per year is calculated based on median cumulative index of 161 years ×µg/g creatinine and median exposure time it results in 12.4 µg/g creatinine/year. If exposure per year is calculated based on mean cumulative index and mean exposure time the result is 17.7 µg/g creatinine/year. No significant changes in tremors were detected in exposed group when compared to the referents.

Frumkin et al. (2001) studied the health effects of long-term mercury exposure on chloralkali plant workers (n=147, male n=137, Female n=10) compared to unexposed workers from nearby workplaces (n=132, male n=117, female n=15). The mean time from the previous exposure at the plant was 5.7 years. Job-exposure matrixes (JEMs) based on historical measurements and personnel data were used to assess exposure. Workplace exposure profiles showed two exposure areas: clean areas of the plant with average exposure levels of 10-20 µg/m^3 and high exposure areas of the plant with average exposure levels of 40-50 µg/m^3. Cumulative exposure stayed below 300 µg/m^3 -years.

Historical data from urine mercury concentration was limitedly available from the years 1988-1991 and the mean urinary mercury concentration was 72.1 µg/l (range 13.0-172.7) corrected to specific gravity of 1.024. Current mean urinary mercury concentration was in exposed workers 2.76 µg/g creatinine (SD 2.04)/3.42 µg/l (SD 2.54) and in unexposed 2.31 µg/g creatinine (SD 1.89)/ 3.12 µg/l (SD 2.48). Performance in qualitative neurological tests (vibration sense, motor speed and coordination, tremor, and one test of cognitive function) of exposed workers were impaired. No changes were detected in renal function. These effects can be interpreted as a result of past exposure rather than current indicating long-term effects of high mercury exposure (Bolt, 2012).

Mathiesen et al. (1999) studied neurological effects in former chloralkali male workers after the end of the exposure. Workers (n=75) exposed to mercury vapor were compared to frequency age-matched referents (n=52). The mean exposure time was 7.9 years (range 1.1-36.2), the mean urinary mercury concentration was 539 (range 41-2921) nmol/(l x year) (corresponding to 80 µg/g creatinine (108 µg/l)), and the mean time after the exposure was 12.7 years (range 1.0-35.0). Current mean urinary mercury concentration was 1.8 nmol/mmol creatinine for exposed and 1.2 nmol/mmol creatinine for referents. Motor function and attention were poorer in the exposed group when compared to the referents. Authors concluded that slight persistent effects were detected on the central nervous system (motor function and attention) after exposure to mercury vapor but not on general intellectual or ability to reason logically.

Ehrenberg et al. (1991) studied the effects of mercury at a thermometer factory. Workers exposed to mercury vapor (n=84) were compared to unexposed workers (n=79). Urinary mercury concentration levels ranged from 1.3-344.5 µg/g creatinine in the exposed group and in the unexposed group concentration was below 10 µg/g creatinine. Personal breathing zone air mercury concentrations were between 25.6 and 270.6 µg/m^3. No significant neurotoxic changes were detected in the exposed group.

Cavalleri et al. (1995) studied color vision in elementary mercury exposed workers (n=33, male n=7, female n=26) who were compared to matched controls (n=33). Urinary concentrations of workers ranged between 28-287 µg Hg/g creatinine. Loss of color vision was detected in workers with urinary concentrations above 50 µg Hg/g creatinine when compared to the controls.

Roels et al. (1985) studied the effects of elemental mercury exposure on changes in hand tremor in male (n=131) and female (n=54) workers. Results were compared to control males (n=114) and females (n=48). Workers were from a mercury-zinc amalgam plant, a chloralkali plant, and two plants manufacturing electrical equipment. Controls were from mercury-free plants located in the same area. Average exposure time was 4.8 years for males and 7 years for females. Mean urinary mercury concentration was 52 µg/g creatinine for males and 37 µg/g creatinine for females. Hand tremor was detected only in male workers. Females' urinary concentrations were mainly below 50 µg/g creatinine and no changes in hand tremor were detected when compared to the control group. Authors suggested a threshold limit value of 50 µg/g creatinine for workers exposed to mercury vapor. In

another study, Roels et al. (1982) found an impairment in psychomotor tests with urinary mercury levels of 50-100 µg/g creatinine although no cut off value was detected. This study was smaller, and it included only 43 workers exposed to mercury vapor and 47 control workers. Controls has never been occupied in the units that uses elemental mercury.

Psychological performance of chloralkali male workers (n=36) was compared to referents (Piikivi et al., 1984). Referents were non-occupationally exposed to mercury. Exposure time was over 10 years. Mean urinary mercury levels of exposed workers was 290 nmol/l (SD 145. 6). Heavily exposed workers had impairment in verbal intelligence test and memory tests. Impairment was seen at time-weighted average urinary concentration levels above 550 nmol/l and/or at peak concentrations above 1500 nmol/l. They also found a dose-response relationship that showed more frequent impairment in intelligence test and memory test above urinary concentrations of 280 nmol/l (corresponding to, 42 µg/g creatinine (56 µg/l)). No information was given on referents urine mercury concentration.

Meta-analysis of Meyer-Baron et al. (2002) studied neurobehavioral effects in mercury exposed workers. This meta-analysis included 12 different studies (Foo et al., 1993; Gonzalez-Ramirez et al., 1995; Günther et al., 1996; Kishi et al., 1993; Kishi et al., 1994; Liang et al., 1993; Mathiesen et al., 1999; Ngim et al., 1992; Piikivi and Hänninen, 1989; Piikivi et al., 1984; Ritchie et al., 1995; Roels et al., 1985; Shapiro et al., 1982; Uzzell, 1988; Uzzell and Oler, 1986), and included 686 workers and 579 controls. The results show decrease of nine significant performance effects (attention, memory, construction and motor performance) for urinary concentrations ranging between 18-34 µg Hg/g creatinine. In addition, dose-response relationship was detected for effect sizes all taken together.

Supporting studies

In addition to these key studies, the following studies were considered to provide supporting data, although were not considered adequate for the guidance value setting.

Kishi et al. (1994) studied long terms effects 18-years after the end of the exposure in mercury miners. Mercury miners (n=76) that had been exposed to mercury concentrations above 1.0 mg/m^3 and had been treated for mercury intoxication were recruited in the study. Miners were compared to age-, sex-, and education-matched referents (n=41) that did not have intoxication. Referents mainly worked in tasks other than mining at the mercury mine. Current mean urine mercury concentration was 3.2 ng/g creatinine for workers and 2.2 ng/g creatinine for referents. Even after the end of the exposure impairment of performance in motor coordination, simple reaction time, and short-term memory was detected in the exposed group when compared to referents. In addition, impairment of hand-eye coordination, tapping, and a color card reading test correlated with duration of exposure. Authors concluded that slight persistent effect on neurobehavioral function was detected even years after the end of exposure in mercury miners.

Soleo et al. (1990) studied the psychological performance of inorganic mercury exposed workers at fluorescent lamp factory. This study included eight chronically exposed workers, 20 occasionally exposed workers and 20 controls. Mean urinary concentrations of chronically exposed workers were 30-40 µg/l (corresponding to 22-30 µg/g creatinine), for occasionally exposed workers 11-20 µg/l, and 5-10 µg/l for controls. Chronically exposed workers had impairment of short-term auditory memory and depression, but no changes in color vision. Because of the limited size of the study, no definitive conclusions can be drawn from it.

Günther et al. (1996) studied neurobehavioral changes in chloralkali electrolysis workers exposed to mercury vapor. During the starting time of the study average exposure time was 12 years and the study lasted for 7 years. Behavioral tests were done four times during the study period. A low exposure group had urine mercury concentration of 21-26 µg Hg/l (n=35-40) and a high exposure group had urine mercury concentrations of 111-152 µg Hg/l (n=14-21). These groups were defined at each of the study points with a cut off value of 53 µg Hg/l. Controls (n= 37-43) were from non-exposed areas of the factory. Performance in finger dexterity, tapping, and aiming was poorer with increasing current exposure of mercury. Authors were not able to study dose-response relationship.

Letz et al. (2000) studied peripheral and central nervous function in former workers of a heavy industrial plant (n=205). Elemental/inorganic mercury exposed workers (n=104) had peak urinary mercury concentration levels of 600 µg/l during their active career. Study was made 30 years after the end of the exposure. Impaired peripheral nervous system function was significantly associated with cumulative mercury exposure. No similar effect on

cognitive function, neurobehavioral test outcomes or dementia was found. Authors concluded that occupational inorganic mercury exposure may have long-term effects on peripheral nervous system even decades after the end of the exposure.

Boogaard et al. (1996) examined natural gas workers exposed to elemental mercury in a retrospective study. The study included a high exposure group (n=18) with median urine Hg concentration of 41 µg Hg/l (range 7-72) (corresponding to 30 µg/g creatinine) during working, a low exposure group (n=22) with median urine Hg concentration of 12 µg Hg/l (range 7-53) during working, and a control group (n=19) median urine Hg concentration of 3 µg Hg/l (range 1-8). Exposure groups (control/low/high) were divided based on the work tasks indicating the exposure level. The median exposure time in the high exposure group was 9 years and the low exposure group 10 years. Neurological testing was carried out after cessation of exposure but time from exposure was not reported. Neurological testing did not show significant correlation between the tremor measures and present nor historical urine mercury concentrations.

Chapman et al. (1990) studied mercury battery workers exposed to mercury vapor. The study group consisted of 18 workers (male n=14, female n=4) and 18 age-matched but not sex-matched controls. The average exposure time was 5.3 years (range 0.3-32) and the mean urinary mercury concentration was 23.1 µg/l (standard deviation 28.3) (corresponding to 17 µg/g creatinine) for exposed workers. No urinary mercury concentration was measured from unexposed controls. Despite absence of neurological symptoms and in the average tremor amplitudes between the groups, statistically significant abnormalities were detected in the tremor frequency distribution. It should be noted that battery workers may be exposed to other toxic substances such as cadmium and lead. However, in this study did not consider other exposures in their study.

A longitudinal study evaluated construction workers four times during two-year period and did not find neurotoxic effects at urinary mercury levels of 1.1-80 µg/l (corresponding to 0.8-59 µg/g creatinine) (Dietz et al. 1997 information from (Triebig and Schaller, 1998).

Table XIa.102: Summary of the critical values in the studies researching neurotoxicity after occupational exposure of mercury

Study	Sample size	Workplace	Endpoint	Cessation time after exposure	Critical value
Key studies					
(Bast-Pettersen et al., 2005)	workers: 49 controls: 49	Chloralkali plant	Tremor	4.8 years (range 4.2-10.0)	NOAEL: 9.3 nmol Hg/mmol creatinine/year (range 4.0–25.4) = 16.5 µg Hg/g Cr/year (range 7.1–45)
(Ellingsen et al., 2001)	workers: 47 controls: 47	Chloralkali plant	Neurological effects, tremor	Study conducted under ongoing work	NOAEL: 9.0 nmol Hg/mmol creatinine/year (range 4.0-19.6) = 16.0 µg Hg/g Cr/year (range 7.1–35)
(Fawer et al., 1983)	workers: 26 controls: 25	Fluorescent tube factory, chloralkali plant, and chemical industry	Tremor	Study conducted under ongoing work	LOAEL: 20.1 (SE 2.1) µg Hg/g creatinine
(Langworth et al., 1992a)	workers: 89 controls: 75	Chloralkali plant	Tremor	Study conducted under ongoing work	NOAEL: 25.4 µg Hg/g Cr (range 0.5-83.3)
(Wastensson et al., 2006, 2008)	workers: 43 controls: 22	Chloralkali plant	Tremor	Study conducted under ongoing work	NOAEL: 5.9 µg Hg/g creatine (range 1.3-25), median 12.4 µg/g creatinine/year,

Study	Sample size	Workplace	Endpoint	Cessation time after exposure	Critical value
					mean 17.7 µg/g creatinine/year
(Frumkin et al., 2001)	workers: 147 controls: 132	Chloralkali plant	Vibration sense, motor speed and coordination, tremor, and one test of cognitive function	5.7 years	LOAEL: Mean 72.1 µg Hg/l during the exposure. Current exposure: 3.42 µg Hg/l (2.76 µg/g creatinine)
(Mathiesen et al., 1999)	workers: 75 controls: 52	Chloralkali plant	Motor function and attention	12.7 years (range 1.0-35.0)	LOAEL: 539 (range 41-2921) nmol/(l x year) (~108 µg/l, 80 µg/g creatinine)
(Ehrenberg et al., 1991)	workers: 84 controls: 79	Thermometer factory	Neurotoxicity	Study conducted under ongoing exposure	NOAEL: 1.3-344.5 µg/g creatinine
(Cavalleri et al., 1995)	workers: 33 controls: 33	Precision instrument factory	Color vision loss	Study conducted under ongoing exposure	LOAEL: 50 µg/g creatinine
(Roels et al., 1985)	workers (m): 131 workers (f): 54 controls (m): 114 controls (f): 48	Mercury-zinc amalgam plant, chloralkali plant, and electrical equipment manufactures	Tremor	Study conducted under ongoing exposure	LOAEL: men 52 µg/g creatinine NOAEL: females 37 µg/g creatinine
(Roels et al., 1982)	workers: 43 controls: 47	Battery production and chloralkali plant	Psychomotor	Study conducted under ongoing exposure	LOAEL: 50-100 µg/g creatinine
(Piikivi et al., 1984)	workers: 32 controls: 36	Chloralkali plant	Intelligence and memory	Study conducted under ongoing exposure	LOAEL: 280 nmol/l (urine) (~56 µg/l, 42 µg/g creatinine)
(Meyer-Baron et al., 2002)	workers: 686 controls: 579	Meta-analysis of different publications	Attention, memory, construction and motor performance	-	LOAEL: 18-34 µg Hg/g creatinine
Supporting studies					
(Kishi et al., 1994)	workers: 76 controls: 41	Mercury mine	Performance in motor coordination, simple reaction time, and short-term memory	18 years	LOAEL: >1.0 mg/m^3 intoxicated workers had neurological symptoms even 18 years after the end of the exposure
(Soleo et al., 1990)	chronically exposed workers: 8	Fluorescent lamp workers	Short term auditory memory, depression,	Study conducted under ongoing exposure	LOAEL: urine 30-40 µg/l (urine) (22-30 µg/g creatinine)

Study	Sample size	Workplace	Endpoint	Cessation time after exposure	Critical value
	occasionally exposed workers: 20 controls: 20				
(Günther et al., 1996)	high exposure: 14-21 low exposure: 35-40 controls: 37-43	Chloralkali plant	Finger dexterity, tapping, and aiming	Study conducted under ongoing exposure	NA
(Letz et al., 2000)	workers: 104 controls: 101	Heavy industrial plant	Peripheral nervous system	30 years	LOAEL: peak 600 µg/l (urine) (~444 µg/g creatinine)
(Boogaard et al., 1996)	High exposure group: 18 Low exposure group: 22 controls: 19	Gas-production site	Tremor	Not reported	NOAEL: 41 µg Hg/l (range 7-72) (~30 µg/g creatinine)
(Chapman et al., 1990)	workers: 18 controls: 18	Cylindrical, magnesium battery, and button type mercury battery plants	Tremor	Study conducted under ongoing exposure	LOAEL: 23.1 µg/l (standard deviation 28.3) (~17 µg/g creatinine)
Dietz et al. 1997 information from (Triebig and Schaller, 1998)	-	Construction site	Neurotoxicity	-	NOAEL: 1.1-80 µg/l (urine) (~0.8-59 µg/g creatinine)

NOAEL= no observed adverse effect level, LOAEL=lowest observed adverse effect level

2.1.5 Kidney toxicity studies

Barregård et al. (1988) studied kidney toxicity on chloralkali workers exposed to mercury (n=41). Results were compared to age-matched controls (n=41). N-acetyl-beta-glucosaminidase (NAG) was increased in mercury exposed workers with urinary mercury concentrations above 4 µg/mmol creatinine (about 50 µg/l: 35 ug/g creatinine). Urinary NAG correlated with urinary mercury levels. No albuminuria was detected in exposed workers with detection limit of 12.5 mg/l.

Cárdenas et al. (1993) studied renal effects of elemental mercury exposure in chloralkali male workers (n=44) compared to controls (n=49). Controls were unexposed workers from the same workplace. The geometric mean urinary concentration of exposed workers was 21.9 µg Hg/g creatinine (31.9 µg/l) and the average exposure time was 11 years (range 1.5-25 years). The geometric mean urinary mercury concentration in controls was 1.6 µg Hg/g creatinine (2.3 µg/l). Cytotoxicity markers showed increased concentration of NAG, brush border markers (BB50, BBA, and HF5), intestine alkaline phosphatase (IAP), and tissue non-specific alkaline phosphatase (TNAP) at mercury concentrations above 50 µg/g creatinine. Biochemical markers prostaglandin E_2, prostaglandin F_2, thromboxane B_2, and glycosaminoglycans decreased at concentrations of ≥50 µg/g creatinine. No changes in functional parameters (creatinine and beta 2-microglobulin in serum, urinary low or high molecular weight proteins) were detected. In addition, no correlation was seen with the exposure time. Authors suggested the changes were reversible and due to recent exposure.

Langworth et al. (1992b) also studied renal effects in chloralkali workers (n=89) versus a control group (n=75). The average exposure time was 13.5 (±8.7 SD) years and the median urinary mercury concentration was 25.4 µg Hg/g creatinine (range 0.5-83.3) in exposed workers. The median urinary concentration in controls was 1.9 µg Hg/g creatinine (range 0-7.6). No differences in the renal dysfunction parameters were detected between the study groups. In the exposed group, there was a trend of increased excretion of NAG and correlation was found between urinary mercury concentration and NAG concentration.

The effects of elemental mercury on renal function was studied in male (n=131) and female (n=54) workers and results were compared to control males (n=114) and females (n=48) (Roels et al., 1985). Workers were from a mercury-zinc amalgam plant, a chloralkali plant, and two plants manufacturing electrical equipment. The average exposure time was 4.8 years for males and 7 years for females. The mean urinary mercury concentration was 52 µg/g creatinine for males and for females 37 µg/g creatinine. Workers had increased urinary concentrations of beta-galactosidase and a retinol-binding protein (RBP) at urinary levels above 50 µg/g creatinine.

Roels et al. (1982) studied kidney toxicity in workers (n=43) exposed to mercury vapor. Workers from battery production and chloralkali plant were compared to a matched control group (n=47). The mean urinary concentrations were 95.5 µg/g creatinine for workers and 1.3 µg/g creatinine for controls. In the exposed workers, an increase in the prevalence of proteinuria and albuminuria was detected when compared to controls.

Alinovi et al. (2002) studied kidney effects in workers (n=122) exposed to inorganic mercury compared to controls (n=197). In the workers group, urinary concentration ranged between 0.1 to 35.0 µg Hg/g creatinine. No differences between the groups were detected in kidney function parameters.

Camerino et al. (2002) compared the renal function of exposed chloralkali workers (n=38) exposed to that of unexposed workers from the same chloralkali plant (n=34). Exposed workers mean urinary mercury concentration was 12 ±8 µg Hg/g creatinine. No changes in renal function parameters were observed.

Ellingsen et al. (2000) studied renal effects in chloralkali male workers after the cessation of exposure to mercury vapor. The study included 47 previously exposed workers and 47 age-matched controls. The cumulative mean urinary concentration was calculated to be 9.0 nmol Hg/mmol creatinine/year (range 4.0-19.6), which corresponds to 16.0 µg Hg/g Cr/year (range 7.1–35). The average exposure time was 13.3 years. The cumulative urinary concentration was based on historical urinary measurements. The current, urinary mercury concentration was 5.9 nmol Hg/mmol creatinine (range 1.1-16.8) corresponding to 10.5 (2.0–30) µg Hg/g creatine. Increased urinary concentration of NAG was detected in workers when compared to the controls but no changes were seen in other kidney parameters.

Buchet et al. (1980) found renal effects in workers exposed to mercury vapor at chloralkali plant (n=63). The average duration of the exposure was 6.8 years. Study participants were divided in four groups based on urinary

mercury concentration: controls (n=88) with urinary mercury concentration below 5 µg/g creatinine, workers (n=37) with urinary concentration of 5-49.9 µg/g creatinine, workers (n=11) with urinary concentration of 50-99.9 µg/g creatinine, and workers (n=15) with urinary concentration above 100 µg/g creatinine. The study detected increased concentrations of high-molecular-weight proteins and beta-galactosidase activity at urinary mercury levels above 50 µg/g creatinine.

Table XIa.103: Summary of the critical values in the studies researching kidney toxicity after occupational exposure of mercury

Study	Sample size	Workplace	Endpoint	Critical value
(Barregård et al., 1988)	workers: 41 controls: 41	Chloralkali plant	Increased excretion of NAG	LOAEL: 50 µg/l= 35 ug/g creat
(Cárdenas et al., 1993)	workers: 44 controls: 49	Chloralkali plant	Increased concentration of NAG, BB50, BBA, and HF5, IAP, and TNAP	LOAEL: 50 µg/g creatinine
(Langworth et al., 1992b)	workers: 85 controls: 69	Chloralkali plant	Renal dysfunction	NOAEL: 25.4 µg Hg/g Cr (range 0.5-83.3)
(Roels et al., 1985)	workers (m): 131 workers (f): 54 controls (m): 114 controls (f): 48	Mercury-zinc amalgam plant, chloralkali plant, and electrical equipment manufactures	Increased urinary beta-galactosidase and a retinol-binding protein (RBP)	LOAEL: 50 µg/g creatinine
(Alinovi et al., 2002)	workers: 122 controls: 137	Not available	No changes in kidney function parameters	NOAEL: 35 µg/g creatinine
(Camerino et al., 2002)	workers: 38 controls: 34	Chloralkali plant	No changes in kidney function parameters.	NOAEL: 35 µg/g creatinine
(Ellingsen et al., 2000)	workers: 47 controls: 47	Chloralkali plant	Increased NAG	LOAEL: 9.0 nmol Hg/mmol creatinine/year (range 4.0-19.6) = 16.0 µg Hg/g Cr/year (range 7.1–35)
(Buchet et al., 1980)	workers: 63	Chloralkali plant	high-molecular-weight proteins and beta-galactosidase activity	LOAEL: 50 µg/g creatinine

NOAEL= no observed adverse effect level, LOAEL=lowest observed adverse effect level

In addition to human studies, several animal studies have been performed to study the nephrotoxicity of inorganic mercury.

EFSA considered the NTP (1993) 6-month sub-chronic rat study to be the key study (EFSA, 2012) for setting a tolerable weekly intake (TWI) for inorganic mercury. This was in line with the assessment by JECFA in 2010 (JECFA, 2010). In this study male and female F344 rats were dosed by oral gavage for 26 weeks at the dose levels of 0, 0.312, 0.625, 1.25, 2.5, and 5 mg mercuric chloride/kg body weight/d. Each dose group included 10 rats of each sex. Kidney weights (absolute and relative) were increased in the dose group of 0.625 mg/kg/d and above. In male rats, the severity of nephropathy increased with increasing dose.

ATSDR determined the study of Apaydin et al. (2016) to be the key study for inorganic mercury. The study was a 28-days oral sub-chronic toxicity study on Wistar rats (n=6/group). Nephrotoxicity was detected at the dose level of 0.015 mg Hg/kg/day of mercuric chloride. No NOAEL could be determined as study included only one dose level in addition to control. They found an impairment of renal function as the serum levels of urea, uric acid, and creatinine were significantly increased in exposed rats. Tubular dilation and glomerular lobulation was found in

the histopathology tests. Also, oxidative stress and peroxidation of lipid membranes was detected. This study has some limitations when compared to the NTP study as the study was only a 28 -day study, with only one dose group and a low number of animals in the exposed group. No information about kidney weights nor quantitative data of histopathological changes is available.

2.1.6 Study limitations

In the occupational studies participant number is typically relatively limited; the sample size in most cases is less than 200. However, there are multiple occupational studies, and one meta-analysis that included over 600 workers. These all help to build a stronger weight of evidence. Additionally, in these occupational studies the exposure characterization and examination of workers have been more detailed than in larger epidemiological studies. Occupational studies are conducted in adult population usually at age of 18-68 (Fields et al., 2017) and typically bigger proportion of participants is males than females or all participants are males. Healthy worker bias is a typical in occupational studies, which may result in the underestimation of health risk. This means that most severely ill workers might have left the workplace and are not included in the studies. In these occupational studies the studied effects are only mild subclinical effects and therefore the risk of healthy worker bias may be less significant.

Most of the occupational studies were carried out in chloralkali workers where elemental mercury is the main toxic chemical. However, there were few studies carried out in battery workers where exposure to other chemicals such as lead, and cadmium may be confounding factors. In the Roels et al. (1985) study exposure to cadmium and lead was studied with biomonitoring and both control and mercury exposed groups had similar levels of cadmium and lead. Chapman et al. (1990) study did not consider other exposures in their study.

Some of the studies had information on biomonitoring available only during the study time or after the exposure period. This could lead to more conservative estimate on the hazardous doses since the exposure may have been higher in the past. However, Bast-Peterson et al. (2005), Ellingsen et al. (2001; 2000) and Wastensson et al. (2006, 2008) had collected biomonitoring data from the whole exposure period and were able to calculate cumulative exposure, thus, exposure assessment can be considered realistic. This exposure characterization is considerably more accurate when compared to epidemiological studies in general where usually spots-samples are conducted for exposure assessment.

2.2 Overview on toxicity refence values

In the ATSDR report the most sensitive target organs and effects were determined for elemental and inorganic mercury (ATSDR, 2024). For elemental mercury with inhalation exposure, neurological effects were found to be the most sensitive effect. The neurological data was based on seven occupational studies (see above: (Bast-Pettersen et al., 2005; Boogaard et al., 1996; Chapman et al., 1990; Ellingsen et al., 2001; Fawer et al., 1983; Langworth et al., 1992a; Wastensson et al., 2006, 2008). From these occupational studies, the lower 95% confidence limit on the weighted mean of 4.92 µg Hg/m^3 for neurological tremor effects was calculated. This calculation resulted in a value of 2.84 µg Hg/m^3. To calculate a minimal risk level an uncertainty factor of 10 was applied, resulting in minimal risk level of 0.3 µg Hg/m^3. Exposure to mercury concentrations in air were converted from urinary levels by applying a steady-state mass balance model.

For inorganic mercury, the most sensitive target organ with oral exposure was in the kidneys (ATSDR, 2024). Data was from animal studies and no NOAEL could be determined, so instead the LOAEL value was applied. The lowest LOAEL of 0.015 mg Hg/kg/day was for subchronic kidney toxicity. ATSDR stated that no epidemiological studies specific for exposure to inorganic mercury salts were identified.

EFSA (2012) recommended a tolerable weekly intake (TWI) value for inorganic mercury based on a 6-month subchronic study in rats. The critical effect was kidney weight change. EFSA used BMD (benchmark dose) modelling to determine lower 95 % confidence limit for a benchmark response of 10 % (BMDL$_{10}$) resulting in 0.06 mg Hg/kg bw/d. An assessment factor of 100 was used and the resulting value was rounded to 4 µg/kg bw per week. EFSA decided to use animal data as human epidemiological studies were case-reports and with the following limitations: a small study group, insufficient control for confounders, inadequate exposure assessment and insufficient differentiation between mercury compounds and routes of exposure.

The German MAK commission (Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area) has determined a biological tolerance value for occupational exposure (BAT) value (the concentration of a substance in the body that does not cause damage to the health in humans) for elemental and inorganic mercury. Based on nephrotoxicity and neurotoxicity data in humans the BAT value of 25 µg Hg/g creatinine corresponding to 30 µg Hg/l was determined (Bolt, 2012; DFG, 2016).

UBA has set HBM-I (the concentration of a substance in human biological material at and below which there is no risk for adverse health effects) and HBM-II values (the concentration of a substance in a human biological material at and above which there is an increased risk for adverse health effects) for elemental mercury and inorganic mercury compounds based on four worker cohort studies (UBA, 1999). An HBM-I value of 5 µg Hg/g creatinine and a HBM-II value of 20 µg Hg/g creatinine were set based on kidney and neurological health effects.

SCOEL recommended a BLV (biological limit value) of 30 µg Hg/g creatinine in urine based on neuro- and kidney toxicity (SCOEL, 2007). It was stated that kidney toxicity and neurotoxicity occur commonly at urinary levels above 35 µg Hg/g creatinine but there was also evidence that neurotoxic effects might already occur at the level of 20-30 µg Hg/g creatinine.

The HBM4EU project set provisional HBM-GV for the general population for inorganic mercury including elemental mercury. The point of departure was the tolerable weekly intake (TWI) value 4 µg/kg bw per week set by EFSA (2012) (Apel et al., 2022). PBPK modelling by INTEGRA was used to derive an HBM-GV of 7 µg Hg/l in urine.

Table XIa.104: Overview of some toxicity reference values

Agency	Substance	Key study	Endpoint	Point of departure (mg/kg bw/d)	Assessment factors	Respective value
External limit/guidance values						
ATSDR (2024), elemental mercury	Elemental mercury	(Bast-Pettersen et al., 2005; Boogaard et al., 1996; Chapman et al., 1990; Ellingsen et al., 2001; Fawer et al., 1983; Langworth et al., 1992a; Wastensson et al., 2006, 2008)	Neurotoxicity, tremor	lower 95 % confidence limit of mean of 7 studies: 2.84 µg Hg/m³	10	0.3 µg Hg/m³.
ATSDR (2024), inorganic mercury	Inorganic mercury	(Apaydın et al., 2016)	Kidney toxicity, sub-chronic	LOAEL 0.015 mg Hg/kg/day	1000	0.01 µg Hg/kg/day
EFSA (2012), general population	Inorganic mercury	(NTP, 1993)	Kidney weight changes in rats	$BMDL_{10}$ of 0.06 mg Hg/kg bw/d	100	TWI 4 µg/kg bw per week
Internal guidance values						
(Bolt, 2012; DFG, 2016) BAT value workers		(Ellingsen et al., 2001; Frumkin et al., 2001; Günther et al., 1996; Kishi et al., 1994; Letz et al., 2000; Mathiesen et al., 1999; Meyer-Baron et	Nephrotoxicity and neurotoxicity	When considering 95 percentile no clinically relevant changes were detected at level of 30 µg/l	-	25 µg Hg/g creatinine corresponding to 30 µg/l

Agency	Substance	Key study	Endpoint	Point of departure (mg/kg bw/d)	Assessment factors	Respective value
		al., 2002) Dietz et al. 1997				
UBA (1999) HBM-I, general population		(Barregård et al., 1988; Cárdenas et al., 1993; Cavalleri et al., 1995; Chapman et al., 1990; Ehrenberg et al., 1991; Langworth et al., 1992b; Piikivi et al., 1984; Soleo et al., 1990)	Kidney and neurotoxicity	No effects detected on levels below 5 µg Hg/g creatinine	-	5 µg Hg/g creatinine
UBA (1999) HBM-II, general population		(Barregård et al., 1988; Cárdenas et al., 1993; Cavalleri et al., 1995; Chapman et al., 1990; Ehrenberg et al., 1991; Langworth et al., 1992b; Piikivi et al., 1984; Soleo et al., 1990)	Kidney and neurotoxicity	35 µg/g creatinine for kidney toxicity and neurological effects 5-50 µg/g creatinine	-	20 µg Hg/g creatinine
SCOEL (2007), workers	Elemental and inorganic mercury	(Meyer-Baron et al., 2002; Piikivi et al., 1984; Roels et al., 1985; Roels et al., 1982)	Kidney and neurotoxicity	35 µg Hg/g creatinine	-	Urine: 30 µg Hg/g creatinine Blood: 10 µg Hg/l
HBM4EU general population (Apel et al., 2022)	Inorganic mercury	(EFSA, 2012)	Kidney weight changes in rats	TWI 4 µg/kg bw per week	PBPK modelling by INTEGRA includes AF of 3.16	7 µg/l in urine

2.3 Choice of the point of departure and derivation of an HBM-GV for workers (elemental and inorganic mercury)

According to the HBM-GV derivation strategy determined by Apel et al. (2020), the first choice to derive HBM-GV is from human data based on a relationship between internal concentrations and health effects. In workers exposed to mercury there are multiple research studies assessing the relationship between the urinary concentrations of mercury and health effects. Workers are exposed to mercury vapors that are mainly derived from elemental mercury although in some occupations workers are additionally exposed to inorganic mercury compounds. For inorganic mercury animal toxicity data is available.

Total mercury in urine is a good long-term exposure biomarker for elemental and inorganic mercury but it cannot specify the difference between the two of them. Methylmercury is limitedly excreted into urine due to resorption in proximal tubule (ATSDR, 2022). Therefore, total mercury in urine is considered relatively specific biomarker for elemental and inorganic mercury. As workers are mainly exposed to mercury vapors, the HBM-GV for workers can be set based on the human data from workers. This HBM-GV covers occupational inhalation exposure to elemental and inorganic mercury.

Several authoritative bodies have determined neurotoxicity and kidney toxicity to be the critical endpoint for elemental and inorganic mercury (Table XIa.104). The main studies assessing the neurotoxicity and kidney toxicity of mercury in occupationally exposed workers have been listed in Table Xla.102 and Table XIa.103. Neurotoxicity has been detected at a mercury concentration in the range of 18-34 µg Hg/g creatinine (Meyer-Baron et al., 2002) and kidney toxicity in the range of 16-50 µg Hg/g creatinine (Alinovi et al., 2002; Barregård et al., 1988; Buchet et al., 1980; Camerino et al., 2002; Cárdenas et al., 1993; Ellingsen et al., 2000; Langworth et al., 1992b; Roels et al., 1982). Kidney effects observed at these concentrations have mainly included increased NAG (N-acetyl-beta-D-glucosaminidase), which is sub-clinical marker providing an early indication of tubular dysfunction resulting from renal disease or nephrotoxic damage. Neurotoxic effects have included clinical effects such as tremor.

Studies detecting tremor as an endpoint did not find effects at arithmetic mean mercury urinary concentration levels of 16-17.7 µg Hg/g creatinine/year (Bast-Pettersen et al., 2005; Ellingsen et al., 2001; Wastensson et al., 2006, 2008). Wastensson et al. (2006, 2008) calculated median cumulative mercury index for the exposure level causing no effects which was 161 µg Hg/g creatinine x years. Considering that the median exposure time was 13 years, this resulted in median exposure of 12.4 µg Hg/g creatinine/year. Occupational biomonitoring data is usually not normally distributed, thus, either a geometric mean or median should be used instead of the arithmetic mean. Neurotoxicity is considered the most sensitive endpoint for elemental and inorganic mercury and 12.4 µg Hg/g creatinine/year is chosen as a point of departure (POD).

As the exposure data is based on several occupational studies with similar conclusions and includes in total hundreds of workers, inter-individual variability is considered by the data and no additional assessment factors are needed. This results in an HBM-GV of 12.4 µg/g creatinine, which is rounded to 12 µg/g creatinine. To convert 12.4 µg Hg/g creatinine into µg/l in urine Equation 1 is used, resulting in 16.9 µg Hg/l which is rounded to 17 µg Hg/l in urine (Santonen et al., 2023). **In conclusion, an HBM-GV for workers is 12 µg Hg/g creatinine and 17 µg Hg/l in urine.**

Equation XIa.1: Conversion from Ug g /creatinine to µg/l (Santonen et al., 2023). Creatinine concentration in 1 liter of urine is approximately 1.36 (Cocker et al., 2011).

$$\frac{\mu g}{l} = \frac{\mu g}{g\ creatinine} \times 1.36\ g\ creatinine/l$$

$$16.9\ \frac{\mu g}{l} = 12.4 \frac{\mu g}{g\ creatinine} \times 1.36\ g\ creatinine/l$$

2.4 Level of confidence attributed to the derived HBM-GV for workers

The level of confidence should be estimated as high, medium, or low when an HBM-GV is derived (Apel et al., 2020; OECD, 2022). The reliability of the data and the calculation method used in the HBM-GV derivation are considered. In this case, the level of confidence for HBM-GV for workers can be estimated to be high as the data is based on correlation between internal exposure and health effects in workers population.

3 HBM-GV for the general population (elemental and inorganic mercury)

Within the HBM4EU project, an HBM-GV for the general population was derived for inorganic mercury including elemental mercury. Derivation was based on a TWI value of 4 µg/kg bw/week set by EFSA. The TWI was based on kidney weight changes and histopathological kidney effects in rats (NTP, 1993). PBPK modelling was used to derive the respective urinary mercury level. The TWI value includes a standard safety factor of 100 that includes inter- and intraspecies variability. No additional safety factor was recommended. This resulted in HBM-GV of 7 µg/l. An animal toxicity study was used as a POD due to lack of epidemiological studies specifically examining inorganic mercury exposure. In the HBM4EU evaluation, it was suggested to perform a new BMD modelling that would combine the NTP study (1993) and the Apaydin et al. (2016) study. In this deliverable, the possibility to perform a new BMD modelling including these two studies was examined. However, the Apaydin et al. (2016) study did not evaluate kidney weight changes in rats, and no quantitative information of histopathological results was given. Thus, BMD modelling was not possible using the study of Apaydin et al. In addition, the Apaydin et al. (2016) study was not carried out according to GLP, it has only one dose group in addition to control, only one sex was studied, and the sample size per group was only six rats.

In the HBM4EU deliverable, the possibility to derive an HBM-GV for the general population for inhalation exposure to elemental mercury was also evaluated. A literature search included general population studies with exposure to dental amalgams as it is shown that dental amalgams releases mercury vapor (ATSDR, 2024; EFSA, 2012; Richardson et al., 2011). It was concluded that there was only limited evidence on the neurotoxicity and kidney toxicity in the general population exposed to dental amalgams. Overall, it was concluded that "the quality and the quantity of data available leading to equivocal results, do not allow deriving an HBM-GV for the general population based on elemental mercury exposure."

3.1 Choice of the point of departure and derivation of the HBM-GV

Although there is not enough evidence from general population epidemiological studies for the basis of the HBM-GV, occupational study data could be used for the derivation of an HBM-GV for the general population.

As a starting point for the derivation of an HBM-GV for general population, inhalation exposure to elemental mercury from the Wastensson et al. study is used. As for the derivation of HBM-GV for workers, a POD of 12.4 µg Hg/g creatinine/year was chosen. As the exposure data is based on internal exposure of occupational studies an additional assessment factor of 2 is used to cover also the vulnerable groups (such as children and the elderly) and the exposure during the whole lifetime. Detailed justification of the assessment factor is presented in the Table XIa.105.

Table XIa.105: Justification of the assessment factor for the general population

	Assessment factor
Interspecies uncertainty factor	1, as the data derivation is based on human data.
Inter-individual assessment factor	2, considering the ratio between the default inter-individual uncertainty factors of 10 in the general population and of 5 in the workers. Considering that human epidemiological data on developmental (neuro)toxicity has not shown clear effects, and in animal studies, developmental and fertility effects have been observed only at higher dose levels when compared to renal effects, no additional factors were applied to cover these effects.
Differences in duration of exposure	1, data is based on chronically exposed individuals.
Dose-response relationship	1, as chosen POD is based on biological values showing no effect.
Quality of whole database	1, there are plenty of data published on the potential effect of mercury. Data includes human data of mercury vapor as well as animal data of inorganic mercury. As urine mercury concentration consist of both elemental and inorganic mercury combination of both substance dataset should be considered. The chosen POD can be considered relatively conservative as it is taken from the study showing the lowest NOAEL, which is 3.6-5.3 µg/g creatinine lower than other studies from the same endpoint. Additionally, the exposure characterization and examination of workers have been more detailed than in larger epidemiological studies.
Overall assessment factor for the general population	2

This results in a value of 6.2 µg Hg/g creatinine that could be rounded to 6 µg Hg/g creatinine. To convert 6 µg/g creatinine into µg/l in urine Equation 2 is used resulting in 7.6 µg Hg/l which is rounded to 7.5 µg Hg/l in urine (Santonen et al., 2023).

Equation XIa.2: Conversion from Ug g /creatinine to µg/l (Santonen et al., 2023). Creatinine concentration in 1 liter of urine is approximately 1.36 (Cocker et al., 2011).

$$\frac{\mu g}{l} = \frac{\mu g}{g\ creatinine} \times 1.36\ g\ creatinine/l$$

$$6.2 \frac{\mu g}{g\ creatinine} \times 1.36\ g \frac{creatinine}{l} = 7.6\ \mu g\ /l$$

The general population is exposed both to elemental and inorganic mercury and the urine total mercury concentration does not differentiate these two species. Mercury vapor is oxidized to Hg^{2+} in the tissues and blood and excreted to the urine as Hg^{2+}(ATSDR, 2024). Therefore, an HBM-GV based on urinary levels should cover both elemental and inorganic mercury exposure.

The calculated value of 6 µg Hg/g creatinine or 7.5 µg Hg/l in urine for mercury vapor is very close to the provisional HBM-GV of 7 µg/l derived for inorganic mercury during the HBM4EU project. HBM-GV for inorganic mercury was based on TWI value determined by EFSA. The TWI value derivation was based on animal data and the most critical endpoint was kidney toxicity. Other target organs were the liver, nervous system, immune system, reproductive and developmental systems. EFSA took in consideration also the reproductive toxicity data in animals as they noted following: "Effects on reproduction have been reported at a low dose ($BMDL_{10}$ for kidney weight) but the study had limitations and the CONTAM Panel did not consider the data sufficiently robust to be used as a basis for establishing a health-based guidance value."

Other reproductive toxicity studies showed effects at three times higher dose than $BMDL_{10}$ of 0.06 mg/kg bw/day. NOAEL of 0.23 mg/kg bw/day based on kidney toxicity was determined and EFSA confirmed also the $BMDL_{10}$ of 0.06 mg/kg bw/day determined by JECFA. This $BMDL_{10}$ was used for TWI derivation and an assessment factor 100 was used to cover remaining uncertainties. The HBM-GV 7 µg/l derived for the inorganic mercury is lower than the value derived for the mercury vapor.

The inhalation exposure of the general population to mercury vapor through dental amalgams has been more prominent than dietary exposure of inorganic mercury. However, due to gradual substitution of amalgam with other dental filling materials during the past couple of decades (starting from 1990's) and finally the ban of the use of mercury in the dental amalgams in EU from the beginning of 2025, it is likely that inorganic mercury would become more prominent exposure source for the general population especially among the younger population. Thus, an HBM-GV should be set on a level that protects from health hazards of both exposure sources: mercury vapor and inorganic mercury. Overall, the provisional guidance value of 7 ug/l derived within the HBM4EU for inorganic mercury can be considered protective also from the effects observed in mercury vapor exposed humans. Therefore, an **HBM-GV of 6 µg Hg/g creatinine (7 µg Hg/l) is proposed for elemental and inorganic mercury in the general population.**

3.2 Level of confidence attributed to the derived HBM-GV for the general population

The level of confidence for the HBM-GV for general population can be estimated to be medium as the data is based on correlation between internal exposure and health effects in workers population. It was not possible to use epidemiological data from the general population for HBM-GV derivation, which can be considered as a limitation decreasing the confidence from high to medium. It is also noted that although potential neurobehavioural effects of elemental mercury has been proposed, there is currently no clear evidence on those effects from epidemiological studies. However, reliability and relevance of the effect supports the medium level of confidence. In addition, medium level of confidence is supported by the fact that the use of human and animal data for the derivation of HBM-GV resulted at the similar HBM-GV level.

4 Discussion

The total urinary mercury concentration measures internal exposure of elemental and inorganic mercury. Workers are mainly exposed to mercury vapors and to a lesser extent to inorganic mercury. Similarly, the general population is more prominently exposed to mercury vapor due to emission of dental amalgams than to inorganic mercury from non-fish dietary products. For the derivation of an HBM-GV for workers and general population, data from occupational studies was used. Neurotoxicity and nephrotoxicity are determined to be the major toxicities of elemental/inorganic mercury. Neurotoxicity was determined to be the most sensitive endpoint and was used as a POD. The Wastenson et al. (2006, 2008) study was used for the derivation of an HBM-GV for workers and the general population.

According to the weight of evidence many studies were showing similar results for the threshold of neurotoxicity. The Wastensson et al. study had the lowest no effect level and this was therefore used as a POD. For workers, an HBM-GV of 12 µg/g creatinine or 17 µg Hg/l from urine was proposed. This value is lower than SCOEL had earlier proposed as a biological limit value for workers (30 µg Hg/g creatinine) (SCOEL, 2007). Proposed HBM-GV for workers is also lower than BAT value of 25 µg Hg/g creatinine (30 µg Hg/l) set by German MAK commission in 2005 (Bolt, 2012; DFG, 2016). Since the SCOEL report and the BAT value evaluation, new studies have been published and contributed to a lower HBM-GV level.

For the general population, there are no suitable epidemiological studies specific for inorganic mercury (ATSDR, 2024). Also, there are not enough epidemiological studies of the general population exposed to mercury vapor through dental amalgams to fully evaluate connection between health effects and urinary concentration and their quality is poor for setting an HBM-GV (Apel et al., 2022). Therefore, occupational studies were used to derive HBM-GV for the general population. As a starting point the same Wastensson et al. study was used as for the derivation of an HBM-GV for workers.

For the general population, an additional assessment factor of 2 was used to cover lifetime exposure and vulnerable groups including children and the elderly. Derivation of an HBM-GV for the general population resulted in urinary concentration of 6 µg/g creatinine or 7 µg Hg/l. The same value was obtained when an HBM-GV was derived for inorganic mercury based on animal data. This value is also in accordance with the German HBM-I value of 5 µg/g creatinine or 7 ug/l (the concentration of a substance in human biological material up to which there is no risk for adverse health effects). When compared to the available biomonitoring data, it can be concluded that general population levels stay generally below this proposed HBM-GV because the highest urinary mercury 95th percentile level has been 6.6 µg/l (Northern France) (Apel et al., 2022).

The HBM-GV for workers and the general population are in line with each other. Exposure time for workers is usually considered to be 40 years whereas in the general population it is lifetime. On the other hand, mercury is a cumulative substance, therefore, the increased body burden obtained during the working life decrease very slowly especially in the critical organs after the working life. Since the general population includes vulnerable groups that are not included in workers, an additional assessment factor for general population was utilized.

Future research should consider the change of the exposure source from elemental mercury to inorganic mercury in the general population due to substitution of dental amalgams with other filling materials. In addition, continuation of the research on long-term exposure to elemental mercury in the different age groups is recommended. Additional studies should also focus on the toxicity of different forms of mercury and the development of new predictive models that account for multiple mechanisms of exposure.

5 Fact sheet of HBM-GVs for elemental and inorganic mercury for the workers and the general population

FACTSHEET for mercury (elemental and inorganic) for the occupationally exposed adults and general population		
Parameter	**Value / Descriptor**	**Comments**
$HBM\text{-}GV_{worker}$ for elemental and inorganic mercury	17 µg Hg/l, 12 µg Hg/g creatinine	
$HBM\text{-}GV_{general\ population}$ for elemental and inorganic mercury	7 µg Hg/l, 6 µg Hg/g creatinine	
Level of confidence	High for worker, medium for general population	
Status	Agreed	
Year of issue	2025	
EC-No.	231-106-7	
CAS No	7439-97-6	
Molar mass	200.59 g/mol	
Route of exposure	Inhalation	
Derivation method		
Key study, Author(s), Year	(Wastensson et al., 2006, 2008)	
Species	Human	
Route/type of study	Occupational epidemiologic study	
Exposure duration	Chronic	
Critical endpoint	Neurotoxicity, tremor	
POD Value	12.4 µg Hg/g creatinine	
Assessment factors	None for workers, 2 for general population	

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Part XIb

Derivation of HBM guidance values (HBM-GVs) for **methyl mercury** for the general population

Technical reference

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Lead Beneficiary/ Responsible AE	NIPH
Contributing Participants	Jonna Weisell-Laitinen/TTL Tiina Santonen/TTL Petra Apel/UBA Lucija Perharič/NIJZ
Responsible author(s)	Helle Katrine Knutsen/NIPH/ hellekatrine.knutsen@fhi.no
Co-authors	Jonna Weisell-Laitinen/TTL Tiina Santonen/TTL Petra Apel/UBA
Internal Reviewers[2]	WP4 co-leaders
External Reviewers[3]	Experts of Finnish, Greek, Irish, Polish, Spanish, Swiss, UK national hubs, Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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[1] PU = Public

PP = Restricted to other programme participants (including the Commission Services)

RE = Restricted to a group specified by the consortium (including the Commission Services)

CO = Confidential, only for members of the consortium (including the Commission Services)

[2] WP/task co-leader(s) from the same WP

[3] Independent Reviewers that should not be part of the task related to the AD/D or part of projects related to the Task

Document history

Version	Date	Reviewer name/Institutions	Short description of changes
version 1	17/01/2025	Helle Katrine Knutsen	First compiled version for review by PARC 4.1.3.1.a partners.
version 2	25/01/2025	Helle Katrine Knutsen	Revised version considering comments provided by PARC partners
Version 3	28/02/2025 and 10/03/2025	Partner: Lucija Perharič/NIJZ Experts from Finnish NH Greek NH Polish NH Spanish NH Swiss NH UK NH	See Appendix of AD 4.3
Version 3	05/03/2025	For approval by P4.1.3.1.a partners	
Final version	12/03/2025	Review of AD 4.3 by EAA and late suggestions for improvement by experts from Irish NH	Suggestions included

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Abstract

The present report sets HBM-GVs for methyl mercury (MeHg) in blood and hair for the general population. MeHg forms from inorganic mercury of natural or anthropogenic origin by bacteria in water and sediments and accumulates in the food chain. The major exposure is from consumption of fish, in particular large predatory fish. The MeHg HBM-GV in blood replaces a provisional HBM-GV for MeHg previously derived by HBM4EU in 2022. HBM-GV for MeHg in hair has not previously been set. The provisional HBM-GV in blood was based on the tolerable weekly intake (TWI) for MeHg set by EFSA in 2012, which had neurodevelopmental toxicity identified in human epidemiological studies as critical effect. Maternal hair Hg was the exposure biomarker. HBM4EU also partly took new evidence since 2012 into consideration.

After critical review of new evidence identified by HBM4EU and more recent additional systematic reviews the present report concludes that the new data made available since 2012 do not provide a better basis for MeHg HBM-GVs than the reference point previously identified by EFSA. The reference point identified by EFSA in 2012 is therefore maintained as basis for HBM-GVs. The HBM-GV for MeHg in hair is set at 3.6 mg Hg/kg hair. The MeHg HBM-GV in blood is set at 7 µg Hg/L blood. Both HBM-GVs account for interindividual toxicodynamic differences (uncertainty factor [UF] 3.2) and the HBM-GV in blood also accounts for an additional UF 2 for variation in the hair to blood ratio. The level of confidence for the HBM-GVs for the general population is considered high.

Key Words

HBM guidance values, HBM-GV, MeHg, methyl mercury, metals

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Authors and Acknowledgements

Authors:

Helle Katrine Knutsen, NIPH;

Jonna Weisell-Laitinen, TTL;

Tiina Santonen, TTL;

Petra Apel, UBA

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Results

The present report concludes that the new data made available since 2012 do not provide a better basis for MeHg HBM-GVs than the reference point previously identified by EFSA. The reference point identified by EFSA in 2012 is therefore maintained as basis for HBM-GVs. The HBM-GV for MeHg in hair is set at 3.6 mg Hg/kg hair. The MeHg HBM-GV in blood is set at 7 µg Hg/L blood. Both HBM-GVs account for interindividual toxicodynamic differences (uncertainty factor [UF] 3.2) and the HBM-GV in blood also accounts for an additional UF 2 for variation in the hair to blood ratio. The level of confidence for the HBM-GVs for the general population is considered high.

Abbreviations and Acronyms

AD	Autism Disorder
ADHD	Attention Deficit Hyperactivity Disorder
ASD	Autism spectrum disorder
Bw	body weight
BTHg	Blood total Hg
CVD	CardioVascular Disease
HBGV	Health-Based Guidance Value
HBM-GV	Human Biomonitoring Guidance Value
IQR	Interquartile Range
HR	Hazard Ratio
IHD	Ischemic Heart Disease
MeHg	Methyl mercury
OR	Odds Ratio
SD	Standard Deviation
RP	Reference Poit
RR	Relative Risk
UF	Uncertainty Factor

1 Introduction

Methyl mercury (MeHg) is among the prioritized substances in PARC. It forms from inorganic mercury of natural or anthropogenic origin by bacteria in water and sediments under anaerobic conditions. As MeHg accumulates in the food chain, MeHg can be found in relatively high concentrations in large predatory fish. However, MeHg can also be present in terrestrial animal products (e.g. hen eggs) if the animals have been fed with fish meal-containing feed. Furthermore, MeHg can be formed in rice fields and contaminate the rice. MeHg is readily absorbed with absorption efficiency exceeding 90%. It binds to sulfhydryl groups and is distributed throughout the body and crosses the blood-brain barrier and placenta. It is primarily eliminated through excretion in bile and undergoes enterohepatic circulation. The biological half-life is approximately 50 to 70 days in humans. Regarding toxicity, MeHg is known to cause developmental toxicity and to be toxic to the central nervous system.

In humans, mercury is most often measured as total Hg in different matrices, most commonly hair or total blood. The majority of MeHg in the blood and hair arises from fish and seafood consumption, and total Hg in whole blood or hair reflects the dietary MeHg intake. As MeHg constitutes a high proportion of total Hg, these matrices are reflective of exposures from fish eating populations. The concentration of MeHg is the largest fraction of BTHg (blood total Hg), constituting on average around 80% (Sogame and Tsukagoshi 2020) and is also the largest fraction of hair total Hg, constituting approximately 90% (Berglund, Lind et al. 2005).

Biomonitoring the concentration of total Hg is a convenient way to obtain knowledge of MeHg exposure both at a population level and at an individual level.

The aim of the present report is to set HBM-GVs for methyl mercury (MeHg) in blood and hair. A provisional HBM-GV for MeHg of 3.5 µg Hg/L blood was previously derived by HBM4EU (Apel et al. 2022). It was based on the TWI for MeHg set by EFSA in 2012 of 1.3 µg Hg/kg bw per week, based on neurodevelopmental toxicity (EFSA 2012). The HBM-GV was stated provisional due to the comprehensive new human study data available since 2012 that was not included in the assessment and because the report could not be commented on again by national hub contact point experts.

The MeHg HBM-GV in the present report builds on the work from HBM4EU, which included unpublished preliminary results from a review within HBM4EU (WP13) on the question: *"Do recent epidemiological studies on mercury and neurodevelopment support the current health-based guidance value as established by EFSA or does new evidence suggest that revision may be needed?"*. The review searched databases (PubMed and Scopus) from January 2012 to June 2021 and included only prospective cohort studies and case-control studies on maternal (prenatal) dietary exposure and neurocognition of the children. The overall outcome of the review of the 43 included studies was that new studies on neurodevelopmental effects published after the risk assessment of MeHg by EFSA in 2012 did not provide basis for derivation of a new health-based guidance value (HBGV).

In the risk assessment of MeHg by EFSA in 2012 (EFSA 2012), neurodevelopmental effects were the critical endpoint. Furthermore, EFSA pointed out that observations related to cardiovascular disease (myocardial infarction, heart rate variability and blood pressure) were of potential importance, but not conclusive.

The reference point used by EFSA to derive a tolerable weekly intake (TWI) for MeHg of 1.3 µg Hg/kg bw per week was the level of Hg in maternal hair, based on results from birth cohort studies in the Faroe Island and the Seychelles, in which Hg in maternal hair was measured. The reference point from these studies was 11.5 mg/kg maternal hair. By using a maternal hair:blood ratio of 250, EFSA calculated that this level in hair corresponded to a concentration of 46 µg Hg/L blood. This concentration in blood was converted into a daily intake of 1.2 µg Hg/kg bw by using a one-compartment toxicokinetic model. A data-derived uncertainty factor of 2 for the hair:blood ratio and a standard UF of 3.2 for toxicokinetic differences were also applied, resulting in a daily intake of 0.188 µg Hg/kg bw. After multiplying by 7, this resulted in a TWI of 1.3 µg Hg/kg bw for MeHg (EFSA 2012).

The U.S. Environmental Protection Agency had previously derived an Oral Reference Dose of 0.1 µg/kg bw per week using mostly the same studies but an additional uncertainty factor of 10 (EPA 2001). This uncertainty factor was not agreed by EFSA, considering that the toxicodynamic intraspecies UF was not needed since the reference point was derived from pregnant women and the developing children were covered by the data.

The literature review from the HBM4EU WP13 concluded that at approximately 1.8 mg Hg/kg hair there is no sign of adverse effects in the reviewed studies and indicated that the EFSA TWI seems to be conservative.

In the present report under PARC, the calculation of the preliminary HBM-GV for MeHg suggested in HBM4EU is critically reviewed, taking into consideration also new information on cardiovascular disease and heart rate variability, which was indicated by EFSA (2012) to be of potential relevance, in addition to neurodevelopment.

2 Materials and methods

The literature review performed by HBM4EU was used as a starting point for the derivation of HBM-GV for MeHg (Apel et al. 2022, Halldorsson 2022).

The findings, including those listed by HBM4EU but not reviewed, were critically evaluated by expert judgement. Additional systematic reviews were identified by PubMed search.

3 Selection of endpoint as basis for HBM-GVs

EFSA (2012) used neurodevelopmental effects as the critical endpoint. EFSA also pointed out that observations related to cardiovascular disease (myocardial infarction, heart rate variability and blood pressure) were of potential importance, but not conclusive. New evidence for neurodevelopmental effects and cardiovascular disease are discussed in sections 3.1 and 3.2, respectively. Literature review results from HBM4EU on other endpoints than neurodevelopment and cardiac disease are only shortly described in section 3.3.

3.1 Neurodevelopment

As explained in the introduction, HBM4EU in WP13 reviewed the evidence base from January 2012 to June 2021 on prenatal MeHg exposure and neurocognition in children, in order to investigate the need for a revision of the EFSA TWI for MeHg from 2012. According to the project leader, the overall outcome of the review of the 43 included studies was that new studies on neurodevelopmental effects published after the risk assessment of MeHg by EFSA in 2012 did not provide basis for derivation of a new TWI (T.I. Halldorsson, personal communication).

An overview of the results of the studies was presented in Figure 5 in the report on exposure-response associations based on pre-existing cohort data in Delivery Report D13.7 in HBM4EU (Halldorsson 2022). For studies reporting blood Hg, the blood value was converted to hair Hg. According to the results in the figure and the text in the report on the reviewed studies, mean maternal hair concentrations up to ~4.0 mg/kg did not show consistent adverse effects, and adverse effects were largely confined to small studies.

The conclusion of the review by HBM4EU is supported by the conclusion from a recent systematic review on prenatal mercury exposure and neurodevelopment up to the age of 5 years (Dack, Fell et al. 2022). The systematic review followed guidelines for systematic reviews and had a pre-published protocol. It included studies with measured prenatal exposure and neurodevelopmental functioning using continuous scales (not diagnoses or symptom measures) in children aged 0-5 years old published up to December 2020. The final review included 32 papers meeting the inclusion criteria, and in these papers neurodevelopmental functioning had been measured by the use of 23 different scales. Of these, 15 papers passed the criteria for high quality. Mean concentrations of Hg in maternal blood ranged from 0.64 to 3.71 µg Hg/L, and mean maternal hair Hg content ranged from 0.3 to 5.7 mg/kg. Meta-analysis was not possible because of the large heterogeneity of study designs and the results were synthesized narratively in six broad domains of neurodevelopmental outcomes. The paper concluded that "based on current evidence, dietary mercury exposure during pregnancy is unlikely to be a risk factor for low neurodevelopmental functioning in early childhood.". The authors also state that if neurotoxic effects from MeHg are more detectable at higher exposure levels, this appears not to have been studied in the age range of 0-5 years.

To sum up, the review by HBM4EU and the systematic review by Dack et al. (2022) do not indicate a need for revision of the reference point selected for deriving the TWI for MeHg which was set by EFSA in 2012 and was based on neurodevelopmental effects in children.

3.2 Cardiac effects

EFSA (2012) stated that it had become evident that the beneficial effects of fish consumption have to be taken into account when studying cardiovascular disease outcomes in relation to MeHg exposure. The autonomic nervous system is a major regulator of the cardiovascular system including heart rate and blood pressure. Therefore, the neurotoxicity of MeHg may be related to cardiovascular effects.

Myocardial infarction

HBM4EU identified papers addressing cardiovascular disease and risk factors for these via a literature search, but only listed the papers and the outcomes since they did not have the capacity to assess the results.

This literature search identified only one paper (Bergdahl, Ahlqwist et al. 2013) on myocardial infarction. This study on women in Gothenburg (Sweden) showed a strong inverse association between serum total Hg and myocardial infarction. The authors explained that this association was confounded by good dental health and/or fish consumption.

Other studies listed by HBM4EU on heart disease endpoints are shortly described in chronological order below (the references were identified by HBM4EU from systematic reviews, and they are not in the reference list of the HMB4EU report):

Daneshmand, Kurl et al. (2016) assessed stroke risk (which is not the endpoint addressed in this section) in the Kuopio Ischemic Disease Risk Factor Study (KIHD) and found no association with hair Hg.

Downer, Martinez-Gonzalez et al. (2017) investigated the risk of cardiovascular disease (CVD) as a nested case-control study in a study on prevention with a Mediterranean diet (PREDIMED). Mercury concentration in toenails was not associated with cardiovascular disease in the study.

Hu, Eccles et al. (2017) addressed self-reported cardiovascular disease in relation to blood selenium (Se) and Hg (geometric mean 7 µg Hg/L) in a cross-sectional study on 2169 Inuit in Canada. The participants were categorized into four exposure groups based on high or low blood Hg or selenium. Results showed that the low Se and high Hg group had a higher prevalence of cardiovascular disease (OR=1.76 for hypertension, 1.57 for stroke, and 1.26 for MI). These results provide evidence that Se may exhibit a protective effect against Hg on cardiovascular disease.

Larsen, Jorgensen et al. (2018) assessed the association of BTHg and cardiovascular disease in 3083 Greenlandic Inuit. The median (IQR) BTHg in men was 19 (1-44) µg Hg/L and in women 15(1-32) µg Hg/L. There was no association between BTHg and incident CVD (HR 0.99, 95%CI 0.99-1.00) and no interaction with sex.

Tajik, Tuomainen et al. (2019) investigated the association of serum long-chain omega 3 polyunsaturated fatty acids and mercury in hair with odds for myocardial ischemia during exercise in 2199 men (342 of these with a history of coronary heart disease) from the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) who performed a maximal symptom-limited exercise test in 1984-1989. Hair Hg (mean [sd] 2.10 [2.12] µg Hg/g) was associated with the occurrence of exercise-induced myocardial ischaemia in the total group (OR 1.18, 95%CI 1.08, 1.30) in a multivariable adjusted model. Among participants without a history of coronary heart disease, the OR was 1.06 (95%CI 0.94, 1.19).

Yao, Steven Xu et al. (2021) classified NHANES participants into high/low exposure groups for several metals based on urinary and blood concentration and the paper has no specific information on the risk of all-cause mortality for Hg.

Also, one systematic review on ischemic heart disease was cited by the report from HBM4EU (Hu, Lowe et al. 2021).

The systematic review by Hu (Hu, Lowe et al. 2021) covers papers from 1966 to October 2019 and for ischemic heart disease (IHD) (including myocardial infarction) 7 papers were included. These 7 papers reported Hg exposure assumed to be MeHg from fish and seafood in hair, blood, serum or toenails. The pooled OR for IHD was 1.21 (95% CI 0.98, 1.50). It is of note that the results in one study (Bergdahl, Ahlqwist et al. 2013) were entered with an increased RR in the forest plot on ischemic heart disease in the review by Hu (Hu, Lowe et al. 2021), although an inverse relationship (hazard ratio 0.85, 1.04 and 0.83 in Q2, Q3 and Q4, respectively in the fully adjusted model) was reported in the original study. A hair to blood ratio of 1/250 was used to convert blood concentration into hair concentrations, and a regression function according to (Ohno, Sakamoto et al. 2007) was used to convert toe-nail concentrations into hair concentrations. Three of the included studies (Bergdahl, Ahlqwist et al. 2013, Hu, Eccles et al. 2017, Larsen, Jorgensen et al. 2018) were published after the risk assessment by EFSA in 2012. The study by Bergdahl, Ahlqwist et al. (2013) measured Hg in serum, which reflects both inorganic and MeHg exposure. Because no conversion factor between serum and hair is available, this study was not included in the dose-response analysis. The dose-response analysis was conducted for ischemic heart disease based on six papers, of which only one was published after 2012, being the cross-sectional study on Inuit population in Canada (Hu, Eccles et al. 2017). The dose-response analysis indicated a J-shaped dose-response curve with a turning point of 1 to 2 µg/g hair, at which the point estimate of the OR started to increase after an initial decrease. The OR became significantly higher than one between 3 and 4 µg/g hair.

To sum up, three more recent studies than those covered by EFSA (2012) were included in the systematic review by Hu et al 2021 on ischemic heart disease. Given the possible misinterpretation of one of the more recent studies (Bergdahl, Ahlqwist et al. 2013), the lack of association in a relatively large study on Greenlandic Inuit (Larsen, Jorgensen et al. 2018) and that the smaller study on Canadian Inuit (Hu, Eccles et al. 2017) showed a RR of 1.16 with 95% confidence interval (0.60, 3.00), the new information is not sufficient as a basis for HBM-GV.

Heart rate variability and blood pressure

Systematic reviews on heart rate variability in all population groups (Gribble, Cheng et al. 2015) or on blood pressure in children and adolescents (Gallego-Vinas, Ballester et al. 2019) concluded there is limited evidence for an association. This was not considered further for setting of a HBM-GV for MeHg.

3.3 Other endpoints

Developmental/prenatal exposure

The results summarized in the HBM4EU report (Apel et al. 2022) on the developmental outcomes sex ratio, respiratory effects, endocrine disrupting properties, immune effects after prenatal exposure are too few and inconsistent to form basis for an HBM-GV.

Many studies on prenatal/developmental exposure and adverse birth outcomes such as decreased birth weight and preterm birth were identified by HBM4EU. In all except one study (which measured MeHg in cord blood), exposure was measured as total Hg in maternal blood, maternal hair, placenta or cord blood. Most of the studies were included in the systematic review by (Dack, Fell et al. 2021), which concluded that *"Overall, our review did not identify strong evidence that mercury exposure leads to impaired prenatal growth."* Another systematic review (Saavedra, Fernández-Recamales et al. 2022) concluded that there are associations with lower birth weight and inconsistent results for birth length.

The results on the developmental outcomes other than neurodevelopmental have not been assessed comprehensively, but based on the preliminary analysis it can be concluded that they cannot form sufficient basis for an HBM-GV.

Postnatal exposure – neurotoxicity other than neurodevelopmental effects

EFSA (2012) did not find the evidence for associations between postnatal childhood MeHg exposure and autism disorder (AD) or attention deficit hyperactivity disorder (ADHD) sufficient to draw conclusions. Postnatal and adult MeHg exposure and association with neurotoxicity (autism, ADHD) was summarized in a table by HBM4EU. Systematic reviews and metanalyses were also referred to (Saghazadeh and Rezaei 2017, Nilsen and Tulve 2020, Sulaiman, Wang et al. 2020).

Yoshimasu, Kiyohara et al. (2014) performed a meta-regression of impacts of prenatal and early infancy exposure to Hg on autism and ADHD in children. The study authors identified two studies (from the US and Canada, published in 2012) on an association between MeHg exposure and ADHD. After correcting the publication bias, the summary OR (95% CI) was calculated to be 1.60 (1.10–2.33) with non-significant heterogeneity (P = 0.26, I2 = 24%) for the random effects model.

Nilsen and Tulve (2020) performed a systematic review and meta-analysis examining the interrelationships between chemical and non-chemical stressors and inherent characteristics in children with ADHD. According to the authors, the heterogeneity between studies was negligible (T2 = 0, I2 = 0%). They reported that the OR for Hg exposure and all ADHD outcomes was 2.68 (2.16–3.19, $p < 0.0001$). They also stated that the small number of studies cannot be overlooked. Meta-analyses with few data are often inaccurate and the data are presented here to drive future research but not to conclude that a relationship has been proven.

The systematic review and metanalysis by (Sulaiman, Wang et al. 2020) with erratum (Sulaiman, Wang et al. 2021) looked at the exposure to aluminum, cadmium, and mercury and autism spectrum disorder (ASD) in children. They indicated that levels of Hg in hair, urine, and blood were all positively associated with ASD. The review by Saghazadeh and Rezaei (2017) was a systematic review and meta-analysis that linked autism and toxic metals, and furthermore, highlighted the impact of country development status.

Overall, results for ADHD and AD appear inconclusive and there is not sufficient basis to derive a reference point (RP).

Three studies in adults, one on Alzheimer's disease, one on autism and one on amyotrophic lateral sclerosis, are not sufficient to provide a basis to derive a RP.

4 Derivation of HBM-GVs for MeHg

Total Hg in blood reflects MeHg exposure and is not a suitable marker for inorganic (elemental) Hg exposure in fish eating populations. Total Hg in hair is also a good biomarker for MeHg exposure. The work by HBM4EU identified numerous studies on associations between Hg exposure and adverse health outcomes. Neurodevelopment was the critical endpoint identified by EFSA (2012) and cardiac autonomy and possibly blood pressure were identified as of potential interest by EFSA. The studies identified by HBM4EU on these endpoints were also covered by systematic reviews presented in the present report. Taking into account the limited resources for a comprehensive review of the data in PARC and considering the neurodevelopmental endpoints covered by the systematic review in HBM4EU (Halldorsson 2022) the reference point derived by EFSA in 2012 for neurodevelopmental effects appears the most suitable RP to derive an HBM-GV for MeHg.

The provisional HBM-GV for MeHg of 3.5 µg Hg/L blood that was previously derived by HBM4EU (Apel et al. 2022) included an additional UF of 2 for uncertainty in hair measurement. However, this extra UF is not considered necessary anymore, given that the determination of Hg in hair can be considered reliable and is believed to reflect MeHg exposure at all exposure levels and is stable over time (EFSA 2012).

4.1 Hair

The reference point used by EFSA was 11.5 mg/kg maternal hair, based on results from birth cohort studies in the Faroe Island and the Seychelles. A standard UF of 3.2 for toxicokinetic differences in the population is considered necessary. No UF for toxicodynamic differences are needed, since total Hg was measured in maternal hair and neurodevelopmental effects were assessed in the children, being the most vulnerable population group. This leads to an HBM-GV for MeHg in hair of (11.5/3.2) 3.6 mg Hg/kg hair for the general population.

4.2 Blood

The reference point used by EFSA was 11.5 mg/kg maternal hair, based on the results from birth cohort studies in the Faroe Island and the Seychelles. Using a maternal hair:blood ratio of 250, this concentration in hair corresponded to 46 µg Hg/L blood. There is a need for an additional UF to cover the uncertainty in the hair:blood ratio. EFSA 2012 (and FAO/WHO 2004) applied a data derived UF of 2 to account for this uncertainty. EFSA (2012, appendix E, Table E1) reviewed the reported hair:blood ratio and stated that there is a high inter-study and inter-individual variation in this ratio. The variation was, however, sufficiently covered by this factor of 2 according to EFSA. A standard UF of 3.2 for toxicokinetic differences is also needed, so in total an UF of 6.4 is applied. This leads to an HBM-GV of 7.2 (46 µg/L/6.4), rounded to 7 µg Hg/L for MeHg in blood.

4.3 Level of confidence of the derived HBM-GVs in hair and blood

Level of confidence should be estimated as high, medium, or low when HBM-GV is derived (Apel, Rousselle et al. 2020). The level of confidence for the HBM-GVs for the general population are considered high as the data is based on internal exposure and health effects in children, which is the most vulnerable population.

4.4 Conclusion

The HBM-GV for MeHg in hair is set at 3.6 mg Hg/kg hair for the general population. The MeHg HBM-GV in blood is set at 7 µg Hg/L blood. The level of confidence for the HBM-GVs for the general population is considered high

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Part XII

Scoping document on PFAS

Technical reference

Work package	**WP4 - Monitoring and Exposure**
Task	**T4.1 - Human Biomonitoring**
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Contributing Participants	Ruth Moeller / LNS / ruth.moeller@lns.etat.lu Maria Torres Toda / LNS / maria.torrestoda@lns.etat.lu Tamar Bermann / MOH IL / tamar.berman@MOH.GOV.IL Helle Katrine Knutsen/NIPH/ hellekatrine.knutsen@fhi.no Petra Apel/UBA/petra.apel@uba.de
Responsible author(s)	Ruth Moeller / LNS / ruth.moeller@lns.etat.lu Maria Torres Toda / LNS / maria.torrestoda@lns.etat.lu Tamar Bermann / MOH IL / tamar.berman@MOH.GOV.IL
Co-authors	Helle Katrine Knutsen/NIPH/ hellekatrine.knutsen@fhi.no Petra Apel/UBA/petra.apel@uba.de
Internal Reviewers[2]	WP4 co-leaders
External Reviewers[3]	Maria Uhl (EAA) / maria.uhl@umweltbundesamt.at Ingrid Hauzenberger (EAA) / ingrid.hauzenberger@umweltbundesamt.at
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1		Authors	First compiled version for review by P4.1.3.1.a partners and in parallel for review in connection with the acceptance of the AD 4.3
Final version	12.05.2025	EAA reviewers	Further revision including the EAA (reviewer of AD 4.3) comments

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Abstract

Perfluoroalkyl substances (PFAS) are human-made fluorinated compounds that are persistent in the environment, widespread and bioaccumulating in both humans and wildlife. PARC includes aligned human biomonitoring (HBM) studies in children, teenagers and adults, as well as targeted studies in pregnant women and newborns including breast milk sampling. There is a need to interpret data that will emerge from the general population HBM studies for the purpose of risk assessment. In HBM4EU, different human health risk assessment approaches were used to determine whether European teenagers are at risk of developing adverse health effects resulting from exposure to PFAS, i.e. the Hazard index approach, the Sum value approach and the RPF approach.

With the ultimate aim to derive recommendations for deriving HBM-GV for PFAS compounds in the scope of PARC aligned studies, the present work represents a scoping paper summarizing the available health-based reference values and international assessments for those PFAS compounds, both for non-cancer endpoints and carcinogenicity. In addition, the working group reviewed animal and epidemiological data that have been highlighted in a recent literature review of several PFAS compounds published by the German Environment Agency (UBA, 2023). Furthermore, the working group reviewed and summarized 43 systematic reviews of which 25 were meta-analyses, published until November 2024.

Based on this, further work is suggested especially for substances that have a relevant contribution to PFAS exposure and where new epidemiological data for the critical effect domains, namely immune system, fertility and reproductive outcomes, cardio-metabolic outcomes, and fetal growth and birth outcomes, became available in recent years (after 2020). Derivation of HBM-GV for the full scope of PFAS compounds measured in the PARC aligned studies may not be justified due to their minor or negligible contribution to exposure. Furthermore, the working group suggests to review the carcinogenicity database in more detail, as well as evidence for causality for increased cholesterol associated with PFOS and PFOA exposure. Efforts may also focus on additional substances, such as including short-chain PFAS that may be prioritized in the PARC targeted HBM studies.

Key Words

HBM guidance values, HBM-GV, PFAS, Perfluoroalkyl substances

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Authors and Acknowledgements

Ruth Moeller / LNS / ruth.moeller@lns.etat.lu

Maria Torres Toda / LNS / maria.torrestoda@lns.etat.lu

Tamar Bermann / MOH IL / tamar.berman@MOH.GOV.IL

We would like to thank the experts who provided us with feedback on this work. Comments were taken into consideration as far as possible for the finalization of this report.

Abbreviations and Acronyms

4:2 FTSA	4:2 Fluorotelomer Sulfonic Acid
6:2 FTSA	6:2 Fluorotelomer Sulfonic Acid
8:2 FTSA	8:2 Fluorotelomer Sulfonic Acid
9CL-PF3ONS	9-chlorofluorotelomer-3-oxo-1-nonene sulfonic acid
ADD	Acceptable Daily Dose
Adona	4,8-dioxo-3H-perfluorononanoic acid
ALT	Alanine Transaminase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area under the curve
bw/d	Body weight per day
BE	Biomonitoring Equivalents
BLOD	Biological Limit of Detection
BMDL	Benchmark Dose Lower Confidence Limit
BMI	Body Mass Index
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CI	Confidence Interval
CO	Confidential, Only for Members of the Consortium (Including the Commission Services)
COVID	Coronavirus Disease
Cmax	Maximum Measured Concentration
CSF	Cancer Slope Factor
DNA	Deoxyribonucleic Acid
DWLV	Drinking Water Limit Values
EFSA	European Food Safety Authority
EGVs	Environmental Guidance Values
EPA	Environmental Protection Agency (USA)
ExCR	Exposure Concentration Ratio
FOSAA	Ethyl Perfluorooctane Sulfonamide
GGT	Gamma-Glutamyl Transferase
GenX	Hexafluoropropylene oxide dimer acid, HFPO-DA
GDM	Gestational Diabetes Mellitus

HI	Hazard Index
HQs	Hazard Quotients
IARC	International Agency for Research on Cancer
IQR	Interquartile Range
IQ	Intelligence Quotient
LOD	Limit of Detection
LOAEL	Lowest Observed Adverse Effect Level
LOQ	Limit of Quantification
MOA	Mode of Action
MRL	Minimal Risk Level
NASEM	USA National Academies of Sciences, Engineering, and Medicine
NCI	National Cancer Institute
NCI's PLCO	National Cancer Institute's Prostate, Lung, Colorectal, and Ovarian Study
NIEHS	National Institute of Environmental Health Sciences
NHANES	USA National Health and Nutrition Examination Survey
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
OEHHA	USA Office of Environmental Health Hazard Assessment
OR	Odds Ratio
PFBA	Perfluorobutanoic Acid
PFDA	Perfluorodecanoic Acid
PFDoDA	Perfluorododecanoic Acid
PFDoDS	Perfluorododecyl Sulfonate
PFDS	Perfluorodecyl Sulfonate
PFHxA	Perfluorohexanoic Acid
PFHxS	Perfluorohexanesulfonic Acid
PFHpA	Perfluoroheptanoic Acid
PFHpS	Perfluoroheptanesulfonic Acid
PFNA	Perfluorononanoic Acid
PFNS	Perfluorinated Nonylsulfonate
PFPeA	Perfluoropentanoic Acid
PFPeS	Perfluoropentyl Sulfonate
PFTrDA	Perfluorotridecanoic Acid

PFTrDS	Perfluorotridecyl Sulfonate
PFUnDA	Perfluoroundecanoic Acid
PFUnDS	Perfluoroundecyl Sulfonate
PLCO	Prostate, Lung, Colorectal, and Ovarian
PoD	Point of Departure
PP	Restricted to Other Programme Participants (Including the Commission Services)
PPAR	Peroxisome Proliferator-Activated Receptor
PXR	Pregnane X Receptor
QA/QC	Quality Assurance/Quality Control
QSAR	Quantitative Structure-Activity Relationship
RAC	Risk Assessment Committee
RCC	Renal Cell Carcinoma
RCR	Risk Characterization Ratio
REACH	Registration, Evaluation, Authorization, and Restriction of Chemicals
RfD	Reference Dose
RFP	Relative Potency Factor
ROS	Reactive Oxygen Species
RPF	Relative Potency Factor
TDI	Tolerable Daily Intake
TG	Triglycerides
Tmax	Time to Maximum Concentration
TWI	Tolerable Weekly Intake
TRV	Toxicological Reference Value
TK	Toxicokinetics
TD	Toxicodynamics
TT4 Level	Total Thyroxine Level (a thyroid hormone)
U.S. EPA	United States Environmental Protection Agency
UBA	Umweltbundesamt (German Environment Agency)
WG	Working Group
WHO	World Health Organization
µg/kg	Micrograms per kilogram
ng/kg	Nanograms per kilogram
ng/ml	Nanograms per milliliter
bw/d	Body weight per day
PNDT	Prenatal Developmental Toxicity
RCC risk	Renal Cell Carcinoma Risk
ExCR	Excess Cancer Risks
T2D	Type 2 Diabetes
homeostasis markers (e.g., HOMA-IR)	Homeostasis Model Assessment of Insulin Resistance
LDL	Low-Density Lipoprotein
H2N3	Influenza A virus subtype H2N3
PIQ	Potential Intakes of Quantities

1 Introduction

Perfluoroalkyl substances (PFAS) are human-made fluorinated compounds that are persistent in the environment, widespread and bioaccumulating in both humans and wildlife. The use of PFAS is wide-spread including diverse industrial and professional applications, as well as consumer products such as non-stick cooking pans and utensils, firefighting foams, packaging products, carpets, and water- and stain-proof textiles (ATSDR, 2018), to name only a few. Biomonitoring studies have indicated widespread exposure in the general population. In HBM4EU from the study population of teenagers (N = 1,957 from eight countries), four PFAS compounds (PFOS, PFOA, PFNA and PFHxS) were quantified in 100%, 98.4%, 85.6%, and 86.4% of blood samples, respectively. An additional 8 PFAS compounds (PFDA, PFHpA, PFHpS, PFHxA, PFPeA, PFUnDA, PFDoDA, and PFBS) were quantified in 62.4%, 26.7%, 45.6%, 27.3%, 19.9%, 50.7%, 4.2% and 4.0% of blood samples, respectively (Richterová et al 2023).

PARC includes aligned studies in children, teenagers and adults. In the framework of PARC, data on 7 PFAS compounds are defined as "PARC aligned studies scope" for analysis in serum in teenagers and adults (PFOA, PFOS, PFNA, PFDA, PFBS, PFHxS, PFHpS), meaning they are to be measured in the PARC aligned studies. An additional 15 compounds are part of the discovery scope in the aligned studies, meaning that data can be collected on a voluntary basis (4:2 FTSA, 6:2 FTSA, 8:2 FTSA, 9CL-PF3ONS, PFHxA, PFHpA, PFDoDA, PFUnDA, PFPeA, PFBA, PFNS, EtFOSAA, MeFOSAA, Adona, and GenX).

PARC includes also targeted studies on PFAS in children and studies in pregnant women, breastmilk and newborns. These studies will start in the second phase of PARC (Y4; May 2025). The substance scope is not defined yet. However, it is expected that also short-chain PFAS will be prioritized for HBM in the urine and breastmilk. One important substance to mention is Trifluoroacetic acid (TFA), a common breakdown product of other larger PFAS, and which is highly persistent and mobile in the environment and measured in ground- and drinking water.

There is a need to interpret data that will emerge from the general population HBM studies for the purpose of risk assessment. As a continuation of the efforts under HBM4EU, our dedicated working group of the PARC project P.4.1.3.1.a_HBM-GV therefore reviews the need and possibilities for derivation or updating of existing HBM-GV for PFAS substances.

2 Background: Risk assessment approaches for PFAS used under HBM4EU

In HBM4EU, different human health risk assessment approaches were used to determine whether European teenagers are at risk of developing adverse health effects resulting from exposure to PFAS. Three approaches for mixture risk assessment of PFAS were used for comparison (see Bil et al., 2023a). They build on different assumptions and come, accordingly, with different uncertainties:

- **Hazard index (HI) approach**, based on effect levels based on immunotoxicity and birth weight reductions in epidemiological studies. The hazard quotient is calculated for each single compound in the mixture based on the exposure level in the numerator (HBM data measured in the HBM4EU project) relative to the effect level (defined as the effect level observed in human epidemiological studies). By summing the hazard quotients (HQs), the HI is calculated and a HI exceeding 1 indicates that a potential risk to human health may exist.
- **Sum values approach**, as used by EFSA for PFOA, PFNA, PFHxS and PFOS (hereafter called 'EFSA-4'). This was based on EFSA group tolerable weekly intake (TWI) of 4.4 ng/kg bw/week, corresponding to a serum concentration of 6.9 ng/mL in women of reproductive age, based on a serum concentration of 17.5 ng/mL in 1-year-old children. In lack of sufficient data on differences in potencies EFSA applied the assumption that the EFSA-4 are equipotent (i.e. RPFs = 1) for immunotoxic effects in humans. The cumulative risk (Risk Characterization Ratio, RCR) is estimated by summing the serum concentrations of the EFSA-4 in each individual and dividing this by the serum concentration in adults that correspond to dietary exposure (6.9 ng/mL) equal to the TWI. EFSA grouped PFAS based on co-exposure and similar toxicity using an internal (toxicokinetic-corrected) whole mixture approach.
- **Relative potency factor (RPF) approach**. For the RPF approach, toxicokinetic models were generated for 10 PFASs to estimate the internal exposure in the male rat at the blood serum level over time. These internal exposures were then used to derive internal RPFs based on liver effects in male rats (Bil et al., 2022). For the HBM4EU exposure biomarkers, this resulted in inclusion of PFHxA, PFOA, PFNA, PFDoDA, PFBS, PFHxS and PFOS in the RPF approach. The RPF approach for mixture risk assessment of PFAS builds on the assumption of dose addition, setting the potency of the index compound PFOA for liver toxicity in rat studies to 1, and expressing the toxicity of the other compounds relative to this as relative potency factors. PFOA equivalent (PEQ) exposures for each PFAS were calculated, summed to obtain the sum PEQ for each individual, then the cumulative risk is estimated by dividing this by the serum concentrations that correspond to chronic dietary exposure similar to the TWI set by EFSA. In combining the RPF approach with the HBM GVs derived from the EFSA risk assessment of sum of 4 PFAS, it is assumed that not only the EFSA-4 cause immunotoxicity in humans but also the other PFASs for which RPFs are derived, and that the potency ranking of PFASs based on liver toxicity in animals can be extrapolated to the potency ranking of human toxicities such as immunotoxicity.

  The working groups notes that for the purpose of risk characterization a comparison of sum PEQ exposures with the EFSA sum value, which is based on equipotency, is not appropriate.

By applying the above approaches, the HI approach resulted in the highest risk estimates, followed by the RPF approach and the sum value approach. Although three different risk assessment approaches were used, the main results presented in the publication by Uhl et al. (2023) and the main results presented in the HBM4EU policy brief for PFAS focused on the sum value approach. Of note, the HBM4EU dashboard presents PFAS data with comparison to the EFSA-4 value and the HBM-I value of the German HBM Commission.

3 Relative abundance of PFAS compounds in HBM studies

The working group addressed the question of whether HBM-GV values should be derived for several or all of the PFAS compounds included in the PARC aligned studies. The PARC aligned studies include 7 compounds in the compulsory set and an additional 15 compounds in the discovery set. Deriving HBM-GV values for all 23 compounds, and applying an HI approach, sum values approach or RPF approach would be challenging.

Published HBM studies to date indicate that 4 PFAS compounds (PFOA, PFOS, PFNA, PFHxS) contribute over 90% of body burden among the measured PFAS. In the EFSA analysis (2020) on HBM studies in adults, relative contribution of PFAS was 64% for PFOS, 16% for PFOA, 5.6% for PFHxS and 5.1% for PFNA. For studies in children, PFOS and PFOA contributed almost the same with 35% and 36.6% of the total, followed by PFNA (8.8%) and PFHxS (6.7%). For the remaining PFAS analytes, detection frequencies were below 60% and contributions could not be established.

In European teenagers participating in HBM4EU, the quantification frequencies of each of the four EFSA-compounds were above 85%: PFOS (100%), PFOA (98.4%), PFNA (85.6%), and PFHxS (86.4%). The geometric means and 95% confidence intervals (CI) were as follows: PFOS [2.13 µg/L (1.63–2.78)], PFOA [0.97 µg/L (0.75–1.26)], PFNA [0.30 µg/L (0.19–0.45)], and PFHxS [0.41 µg/L (0.33–0.52)]. Additionally, eight other compounds were quantified in some of the European regions, though geometric means and contributions were not calculated in the publication. The quantification frequencies in European teenagers for these additional compounds were as follows: PFDA (62.4%), PFHpA (26.7%), PFHpS (45.6%), PFHxA (27.3%), PFPeA (19.9%), PfUnDA (50.7%), PFDoDA (4.2%), and PFBS (4.0%) (Richterová et al., 2023). Additionally, the working group assessed the relative contributions of each PFAS compound in every individual study included in the HBM4EU. For the teenage population, nine countries participated: Sweden, Norway, Slovakia, Spain, Slovenia, Greece, France, Germany, and Belgium. The relative contribution was calculated by considering the LOQ and the quantification frequencies, which varied across the countries. For the overall contribution in HBM4EU, the working group took into account the weight of each study, based on its sample size.

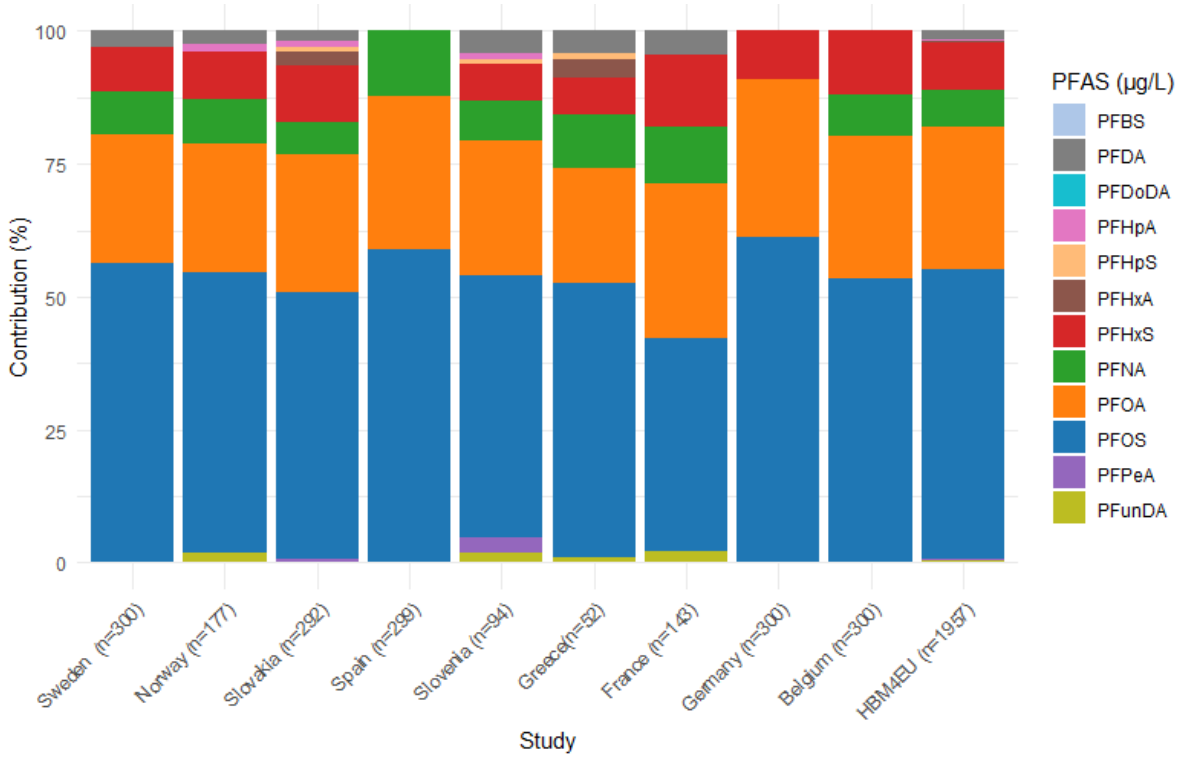


Figure XII.1: Relative Contribution of individual PFAS Compounds in European adolescents, based on median concentrations in blood.

In the HBM studies on adults (EFSA, 2020), PFDA contributed 2.5%, a contribution similar to that observed in adolescents in the HBM4EU (1.5%).

The time trend, in which a limited number of compounds (such as PFOA, PFOS, PFNA, and PFHxS) contribute to large percentage of the overall body burden, is expected to change over time due to regulatory restrictions on use of these compounds. However, given their long environmental and biological half-life, it is likely that these compounds continue to dominate in the body burden of the European adult and adolescent population. Non–target and suspect screening studies for PFAS do not provide insight regarding relative occurrence of PFAS compounds in biological samples in the general population. PFOS was the dominant compound in all participating countries. However, the distribution of PFAS showed some variability depending on the country, with minimal contribution to the overall presence of PFAS compounds.

4 Available health-based reference values and assessments

Several regulatory bodies, expert committees or initiatives have reviewed the toxicological profile of PFAS mixtures or individual substances with a view to support human health risk assessment by deriving guidance values, either as delivered dose or serum/blood level. The following chapters present an overview of work published in recent years.

The compiled overview considers both non-carcinogenic endpoints (chapter 3.1) as well as carcinogenicity (chapter 3.2). The emphasis of the working group was on identifying reviews that have been published after the extensive review of EFSA (2020).

4.1 Non-carcinogenic endpoints - reference values

The tables below present the identified health-based reference values that are based on human data for non-carcinogenic effects (incl. guidance values expressed as serum/blood concentration and reference doses as delivered dose), and reference doses for non-carcinogenic effects based on animal data (either converted to human equivalent serum/blood concentrations or as delivered dose).

Table XII.1: Health-based guidance values based on human data - non-cancer effects

Non-Cancer - Human data derived risk values (based on serum or blood levels)		
Compound/value	**PoD**	**Details**
U.S. EPA (2024) **PFOA** **RfD of 3 x 10^{-8} mg/kg bw/d**	**RfD based on human data.** Co-critical oral RfD of 0.03 ng/kg bw/d based immunological, developmental and cardiovascular effects: Immunological effects (↓**serum IgG**)): **1.24-3.47 ng/mL** (BMDL0.5SD) (3.47 ng/mL for anti-diphtheria antibody concentrations in children (Budtz-Jørgensen and Grandjean, 2018); Developmental effects (↓**Birth weight**): **1.2-9.1 ng/mL** (BMDL5RD) (2.2 ng/mL for decreased infant birth weight by Wikström et al., 2020); Metabolic effects (↑**Cholesterol**; increasing risk for cardiovascular effects): **2.29-5.28 ng/mL** (BMDL0.5SD -BMDL5RD. (2.29 ng/mL for increased total cholesterol in adults by Dong et al., 2019). Hepatic effects (↑ALT)**: 3.76-66.0 ng/mL** (BMDL5RD) Total UF of 10 to account for intraspecies variability.	EPA (2024): FINAL Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts. The final assessment incorporates peer-reviewed studies captured from: EPA's 2016 PFOA HESD (U.S. EPA, 2016c), literature searches of scientific databases and gray literature from 2013 through February 2023.

Non-Cancer - Human data derived risk values (based on serum or blood levels)		
Compound/value	**PoD**	**Details**
U.S. EPA (2024) **PFOS** **RfD of 1 × 10^{-7} mg/kg bw/d**	**RfD based on human data.** RfD of 1×10^{-7} mg/kg bw/d. Candidate RfDs within the developmental and cardiovascular outcomes are the same value (i.e., 1×10^{-7}mg/kg/day). The available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOS exposure. **Developmental effects**: **7.7 ng/mL** (Wikstroem 2020) Metabolic effects (↑**Cholesterol**; increasing risk for cardiovascular effects): **9.34 ng/ml** (Dong et al. 2019) Total UF of 10 to account for intraspecies variability.	EPA (2024): FINAL Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts. The final assessment incorporates peer-reviewed studies captured from: EPA's 2016 PFOS HESD (U.S. EPA, 2016b), literature searches of scientific databases and gray literature from 2013 through February 2023,
CalEPA (2024) **PFOA** **ADD = 0.87 ng/kg bw/d**	**ADD based on human data.** OEHHA selected the **NOAEC of 9.8 ng/ml** for **elevated ALT** from the Gallo et al. (2012) study as the PoD for its PFOA Acceptable Daily Dose (ADD) calculations, using clearance factor of 0.28 ml/kg-day, with an UF of √10 for intraspecies variation. ADD = (POD × CF) ÷ UF = (9.8 ng/ml × 0.28 ml/kg-day) ÷ √10 = 0.87 ng/kg-day For PFOA, the lowest animal PoD was not retained due to lack of serum levels, an appropriate kinetic model, furthermore the complexity of exposure scenario (developmental study). Gallo (2012): "Serum Perfluorooctanoate (PFOA) and Perfluorooctane Sulfonate (PFOS) Concentrations and Liver Function Biomarkers in a Population with Elevated PFOA Exposure". While the Gallo (2012) study does not provide the lowest PoD several advantages were noted with this study and thus preferred over human studies on immunotoxicity (such as Abraham 2020).	OEHHA presents a detailed assessment of PFOS and PFOA association with increased cholesterol, concluding (chapter 5.3.4) "*in summary, the current epidemiologic literature provides evidence that PFOA and PFOS can cause increased total cholesterol in humans… In contrast, some animal studies have shown decreased cholesterol with PFOA and PFOS exposure. Different results in animals and humans may be explained by the stronger activity of PPARα in animals, which is involved in the metabolism of cholesterol and fatty acids.*" Literature review until September 2019. OEHHA, 2024: Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water.
CalEPA (2024) **PFOS** **ADD of 0.64 ng/kg bw/d**	**ADD based on human data** OEHAA selected the **LOAEC of 16.4 ng/ml** from the Steenland et al. (2009) study of PFOS and elevated Total **Cholesterol (TC)** in adults from the C8 study area as the basis of its ADD calculations. ADD = (POD × CF) ÷ UF = (16.4 ng/ml × 0.39 ml/kg - day) ÷ √10 = 0.64 ng/kg bw/d	OEHHA presents a detailed assessment of PFOS and PFOA association with increased cholesterol, concluding (chapter 5.3.4) "*in summary, the current epidemiologic literature provides evidence that PFOA and PFOS can cause increased total cholesterol in humans… In contrast, some animal studies have shown decreased cholesterol with PFOA and PFOS exposure. Different results in animals and humans may be explained by the stronger*

Non-Cancer - Human data derived risk values (based on serum or blood levels)		
Compound/value	**PoD**	**Details**
	ADDs based on animal data were not calculated for PFOS due to the availability of human data for derivation of a health-protective concentration. Steenland (2009): Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant.	*activity of PPARα in animals, which is involved in the metabolism of cholesterol and fatty acids."* Literature review until September 2019. OEHHA, 2024: Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water.
German HBM Commission (Umweltbundesamt 2016/2018; Hölzer et al., 2021; Schümann et al. 2021). **PFOA** **HBM-I = 2 ng/ml plasma** **PFOS** **HBM-I = 5 ng/ml plasma**	**PFOA** **HBM-I: 2 ng/ml plasma** HBM-II: 5 ng/ml (women at child-bearing age) HBM-II: 10 ng/ml (all other groups) **PFOS** **HBM-I: 5 ng/ml plasma** HBM-II: 10 ng/ml (women at child-bearing age) HBM-II: 20 ng/ml (all other groups) Significant contrasts were reported for human blood plasma concentrations between 1 and 10 ng PFOA/ml, and 1–15 ng PFOS/ml, respectively. HBM-I-value represents the concentration of a substance in human biological material below which there is no risk for adverse health effects. HBM-I is derived based on compilation of relevant PoD across several adverse effects, **no additional AFs applied**. HBM Commission rated effects in the following areas as well proven, relevant, and significantly associated with exposure to PFOA and/or PFOS: fertility and pregnancy, weights of newborns at birth, lipid metabolism, immunity, sex hormones and age at puberty/ menarche, thyroid hormones, onset of menopause.	HBM-I and –II are PFAS levels without or with effects in epidemiologic studies. They are not consistent with an equivalent to health-based guidance values (e.g. TDI, RfD). The HBM-I value derived here represents, from a precautionary perspective, the limit value between the range of values where no adverse health effects are expected and the range of values where more in-depth monitoring (and, if appropriate, preventive advice aiming at a reduction of exposure) is required. HBM-II value (established 2019) corresponds to the concentration in human biological material which, when exceeded, may lead to health impairment Evaluation state as of July 2015/May 2016 and follow-up research until May 15, 2017.

Non-Cancer - Human data derived risk values (based on serum or blood levels)		
Compound/value	**PoD**	**Details**
EFSA (2020) **TWI = 4.4 ng/kg bw/week** **(corresponding critical serum level:** **(PFOA+PFNA): 2 ng/mL** **(PFOS+PFHxS): 4.9 ng/mL** **(PFOS+PFNA+PFOS+PFHxS; EFSA-4): 6.9 ng/mL)**	TWI 4.4 ng/kg bw/week (**no additional AF**) derived for EFSA-4 as sum value. EFSA-4 serum concentration of **17.5 ng/mL in children** of 1-year-old (BMDL10), corresponding to long-term **maternal exposure of 0.63 ng/kg bw per day**, resulting in **EFSA-4 serum level in the mother at 35 years of age of 6.9 ng/mL**. Based on the mean levels of these PFASs in 1-year-old breastfed infants of 17,5 ng/mL (Abraham et al., 2020), relative contributions of 48.4, 1.7, 6.1 and 43.8% were calculated, equal to 8.47, 0.30, 1.06 and 7.67 ng/mL, respectively for PFOA, PFNA, PFHxS and PFOS, in the children, used to calculate corresponding critical serum levels in mothers and related daily intakes: PoD is risk factor for disease rather than a disease, and since the study was based on infants, which appear to be a vulnerable population group, no additional UFs for potential intraindividual differences in toxicokinetics and toxicodynamics were deemed necessary. In the previous Opinion (EFSA 2018), the CONTAM Panel used the effects on serum cholesterol levels to derive TWIs for both PFOS and PFOA: PFOA: TWI = 6 ng/kg bw/d, PoD = BMDL05 = 9.2-9.4 ng/ml serum, corresponding to 0.8 ng/kg bw/d. PFOS: TWI = 13 ng/kg bw/d; PoD = BMDL05 = 21-25 ng/ml serum, corresponding to 1.7-2.0 ng/kg bw/d (1.8 ng/kg bw/d chosen weighing overall evidence serum cholesterol, antibody response after vaccination, and birth weight). Although the association with increased cholesterol was observed in a large number of studies, the CONTAM Panel (EFSA 2020) considers the uncertainty regarding causality larger, primarily due to a postulated biological process around the enterohepatic cycling of both PFASs and bile acids, the latter affecting serum cholesterol levels (confounding).	EFSA did not specifically determine acceptable serum PFAS levels, however 6.9 ng/mL serum concentration could be considered a serum level for **women of reproductive age** below which risk in her breastfed child is negligible. Literature review until August 2019.

Table XII.2: Health-based guidance values based on animal data - non-cancer effects

Non- Cancer - Animal data derived risk values		
Compound/value	**PoD**	**Details**
ATSDR (2021) $MRL_{intermediate}$: **PFOA: $3x10^{-6}$ mg/kg bw/d** **PFOS: $2x10^{-6}$ mg/kg bw/d** **PFHxS: $2x10^{-5}$ mg/kg bw/d** **PFNA: $3x10^{-6}$ mg/kg bw/d**	**$MRL_{(intermediate)}$ derivation based on animal data**. PFOA: Skeletal effect mice ($LOAEL_{HED}$ 0.000821 mg/kg bw/d (AF = 300); Koskela et al., 2016) PFOS: Delayed eye opening and decreased pup weight in rats ($NOAEL_{HED}$ 0.000515 mg/kg bw/d (AF = 300)(Luebker et al., 2005) PFHxS: Thyroid follicular epithelial hypertrophy/hyperplasia in rats ($NOAEL_{HED}$ 0.0047 mg/kg bw/d (AF = 300); Butenhoff et al., 2009) PFNA: Decreased body weight and developmental delays in mice ($NOAEL_{HED}$ 0.001 (AF = 300); Das et al., 2015).	Human data were reviewed but not used for MRL derivation. No MRL was derived for **chronic exposure duration because animal data considered insufficient.** Following substances were reviewed: PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoDA, PFBS, PFHxS PFOS, and FOSA. Literature review until November 2018.
US EPA (2021) **PFBS** **Chronic RfD (free acid) = 3×10^{-4} mg/kg bw/d** (rounded) or 0.00028 mg/kg bw/d K+-PFBS Chronic RfD = 3×10^{-4} mg/kg bw/d (rounded) or 0.00032 mg/kg bw/d	**Chronic RfD (low confidence) and Subchronic RfD (medium confidence) based on animal data**. PNDT study in mice with NOAEL of 50 mg/kg bw/d (thyroid effects); PoD_{HED} = 0.095 mg/kg bw/d (AF = 300). Because of the lack of studies that specifically evaluated health effect domains of emerging concern across the PFAS class (immunotoxicity, mammary gland development, or neurodevelopmental) at any exposure duration, but particularly for chronic duration the confidence in the database specifically for a chronic RfD is low. Sub-chronic medium-confidence RfD derived; PoD_{HED} = 0.095 mg/kg bw/d (AF = 100); Subchronic RfD = $1x10^{-3}$ mg/kg bw/d for K+PFBS and $9x10^{-4}$ mg/kg bw/d PFBS.	Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3); 2021
US EPA (2021) **GenX** **Chronic RfD = $3x10^{-6}$ mg/kg bw/d** or 0.003 µg/kg bw/d	**Chronic RfD derived based on animal data.** Oral reproductive/developmental toxicity study in mice with a NOAEL of 0.1 mg/kg/day based on liver effects (a constellation of lesions, including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) in females; PoD_{HED} = 0.01 mg/kg bw/d (AF = 3000) (DuPont-18405-1037, 2010; NTP, 2019).	Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3); 2021

Non- Cancer - Animal data derived risk values		
Compound/value	**PoD**	**Details**
HBM4EU (2020) **The derivation of a single value for PFHxS is not considered adequate at this stage**.	**HBM-GV derivation based on animal data.** In view of the **uncertain data situation and the mostly co-occurrence of PFHxS and other perfluorinated substances** in the environment and in humans, **the derivation of a single value for PFHxS is not considered adequate at this stage**. Based on animal data (Chang et al. (2018): NOAEL (mice, 42 days) = 0.3 mg/kg bw/d (reproscreen) corresp. to 50 µg PFHxS/ml x AF (6 (time) x 4.5 (TD) x 10 (intraspecies) x 3 (database)) = 0.111 µg/PFHxS/ml.	Substance dossier on Perfluorohexane sulfonic acid (PFHxS) prepared under HBM4EU (not published for consultation)
Dr. Voss on behalf of UBA (2020) **PFBS** **Provisional HBM-I = 1 mg PFBS/l serum**	**HBM-I and HBM-II (provisional) based on animal data.** Provisional HBM-I = 1 mg PFBS/l serum and the proposed provisional HBM-II is 3.4 mg PFBS/l. Basis: subchronic study with rats (Lieder et al., 2009a); (NOAEL 51 mg PFBS/(kg bw x d) (Haematology, rat, converted to PFBS); AF = 2 (Time) x 156 (TK) x 2.5 (TD) x 10 (intraspecies). HBM based on developmental study in mice led to much lower values: HBM-I = 3 ng/ml; HBM-II = 13 ng/ml. These values would be in the range of those discussed by the HBM Commission in the assessment of PFOS (Hölzer et al., 2015) or the HBM-I value of 5 ng/ml blood plasma presented by the Commission (HBM-Kommission, 2016) and would thus indicate a potency for PFBS comparable to that of PFOS. However, in view of the limited data available on toxicokinetics in mice and the above-mentioned further uncertainties regarding the transferability of the effects on thyroid hormones from mice to humans, a derivation on this basis was considered to be too uncertain overall.	In view of the uncertainties regarding the toxicokinetic data used as a basis, the mechanistic transferability of the findings in rodents to humans, and the overall limited data available, it is proposed that the values be shown as "provisional".
Dr. Voss on behalf of UBA (2020) **PFBA** **Provisional HBM-I = 0.1 mg PFBA/l serum**	**HBM-I and HBM-II (provisional) based on animal data.** Provisional HBM-I = 1 mg PFBS/l serum and the proposed provisional HBM-II is 0.5 mg PFBS/l. Basis: subchronic study in rats (Butenhoff et al., 2012); (NOAEL 6 mg PFBA/(kg bw x d) (thyroid gland, liver, rat); AF = 2 (Time) x 8 (TK) x (2.5 (TD) x 10 intraspecies)	In view of the uncertainties regarding the toxicokinetic data used as a basis, the mechanistic transferability of the findings in rodents to humans, and the overall limited data available, it is proposed that the values be shown as "provisional".

Non- Cancer - Animal data derived risk values		
Compound/value	**PoD**	**Details**
	Basis: Developmental toxicity study in mice (Das et al., 2008); LOAEL 35 mg PFBA/(kg bw x d) (developmental toxicity, mouse); AF = 3 (LOAEL-NOAEL) x 27 (TK) x 2.5 (TD) x 10 (intraspecies)	
Dr. Voss on behalf of UBA (2020) **PFHxA** **Provisional HBM-I = 0.5 mg PFHxA/l serum**	**HBM-I and HBM-II (provisional) based on animal data.** Provisional HBM-I = 0.5 mg PFHxA/l serum and the proposed provisional HBM-II is 1.74 mg PFHxA/l. Basis: chronic study in rats (Klaunig et al., 2014); NOAEL 30 mg PFHxA/(kg bw x d) (kidney, female rats); AF = 1 (time) x 43 (TK) x 2.5 (TD) x 10 (intraspecies)	In view of the uncertainties regarding the toxicokinetic data used, in particular the elimination kinetics of PFHxA in humans, and the overall limited data available, it is proposed by that the values should be considered "provisional".
Health Canada (2018) **PFOA** **TDI = $2.1x10^{-5}$ mg/kg bw/d**	**TDI based on animal data.** TDI = 21 ng/kg bw/d based on BMDL10 of 0.05 mg/kg bw/d in male rats and human equivalent PoD of 0.521 µg/kg bw/d (composite AF = 25) (13 weeks rats study, Hepatocellular hypertrophy)	More recently, PFAS were re-reviewed by Health Canada, but no reference values were derived: 2024: OBJECTIVE FOR PER- AND POLYFLUOROALKYL SUBSTANCES The new sum PFAS drinking water objective is not health-based and ALARA is recommended. 2023: DRAFT State of Per- and Polyfluoroalkyl Substances (PFAS) Report
Health Canada (2018) **PFOS** **TDI = $6x10^{-5}$ mg/kg bw/d**	**TDI based on animal data.** TDI = 60 ng/kg bw/d based on NOAEL of 0.021 mg/kg bw/d for hepatocellular hypertrophy in rats; PoD_{HEQ} = 1.5 µg/kg bw/d (composite AF = 25) Butenhoff et al., 2012 (104 weeks feeding study rats)	More recently, PFAS were re-reviewed by Health Canada, but no reference values were derived: 2024: OBJECTIVE FOR PER- AND POLYFLUOROALKYL SUBSTANCES The new sum PFAS drinking water objective is not health-based and ALARA is recommended. 2023: DRAFT State of Per- and Polyfluoroalkyl Substances (PFAS) Report

Further to the above, based on PFOA guidance values derived by U.S. EPA (draft, 2023), **Tewlik et al. (2023)** derived Biomonitoring Equivalents (BE) of 0.2 ng/ml PFOA for women of reproductive age (low birth weight, vaccine response), and of 0.8 ng/ml (Cholesterol) for the general adult population. Based on the MRL derived from ATSDR, an equivalent of 6-10 ng/ml (MRL) was derived. Starting from the points of departure, the group employed pharmacokinetic data/models and uncertainty factors. Points of departure in pregnant rodents (U.S. EPA 2016, ATSDR) were converted into fetus and pup serum concentrations using an animal gestation/lactation pharmacokinetic model, and equivalent human fetus and child concentrations were converted into BEs in maternal serum using a human gestation/lactation model. For epidemiology-based guidance values (U.S. EPA 2023, draft), BEs were directly based on epidemiological data or derived using a human gestation/lactation pharmacokinetic model.

The **NASEM committee (2022)**, funded by ATSDR and the National Institute of Environmental Health Sciences (NIEHS), in its guidance on PFAS exposure, testing and clinical follow-up recommended applying the approach of weight-based dose additivity using HBM Commission's values, there by deriving the following levels: **< 2 ng/ml** not expected to cause adverse effects; **2-20 ng/ml** associated with a "potential for adverse effects", and **> 20 ng/ml** associated with "an increased risk of adverse effects". NASEM based their assessment on EFSA (2020) and German HBM values; the lowest PFAS risk-based level being 2 ng/mL (HBM-I for PFOA), and the highest PFAS level being 20 ng/mL (HBM-II for PFOS in the general population), demonstrating that risks are unexpected below 2 ng/mL and that the risk of PFAS-associated effects at the population level is increased at 20 ng/mL. Thus, the risk-based levels for sensitive populations fall between 2 ng/mL and 20 ng/mL. Mixture assessment was considered appropriate, while the EFSA approach being simple to apply and has been used in other regulatory mixture efforts, it is only covering 4 PFAS. Due to evidence suggesting many PFAS having similar toxic effects (Kwiatkowski et al., 2020), the committee believed the additive approach could be applied to the PFAS being analysed in the NHANES (MeFOSAA, PFHxS, PFOA [linear and branched isomers], PFDA, PFUnDA, PFOS [linear and branched isomers], and PFNA). It is noted that the NASEM recommendation does not take into account relative contributions of PFAS analysed in NHANES.

4.2 Literature review and assessment for PFAS (German Environment Agency)

In addition to the above presented assessments, the **German Environment Agency (UBA, 2023)** published a literature review and assessment of available toxicological data for PFAS. The aim of this review was the evaluation of PFAS by carrying out literature searches on toxicological and epidemiological data that can be used as basis for the derivation of drinking water limit values (DWLV). Substances in scope were those of the Directive (EU) 2020/2184 "sum of PFAS" parameter defined as the sum of 20 perfluorinated carboxylic and sulfonic acids with a chain length of 4 to 13 carbon atoms with a parameter value of 0.1 µg/l in drinking water. This includes the following PFAS: 1) PARC aligned study harmonized scope: PFDA, PFBS, PFHpS and the EFSA-4 (PFOA, PFNA, PFHxS and PFOS), 2) PARC aligned studies discovery scope: PFBA, PFPeA, PFHxA, PFHpA, PFUnDA, PFDoDA and PFNS; 3) substances not prioritized in PARC aligned studies: PFTrDA. PFPeS, PFDS, PFUnDS, PFDoDS, and PFTrDS.

For the 7 PFAS which were already assessed by the UBA in its previous drinking water 2016/2017 report, the search was limited to studies from 2017 onwards, while for the PFAS-4 substances evaluated by EFSA in 2020, the time frame of the search was limited to studies published 2020 onwards. The working group has reviewed the conclusions and recommendations for substances included in the aligned studies, including Klimisch score evaluation. The following table presents the outcome of the literature review.

Table XII. 3: Overview of proposed PoD and assessment factors to derive reference doses for the purpose of German Drinking Water Limit Values

Studies identified by UBA (2023)				
RfD **PoD / AF**	**Study**	**Toxicological effect**	**Klimisch**	**Overall database and remarks**
PFDA 0.01 µg/kg bw/d NOAEL = 0.125 mg/kg bw/d; **AF = 12600** (6 (time) x 210 (interspecies: 84 (TK) x 2.5 (TD)) x 10 (intraspecies))	4-weeks oral gavage rat study; Frawley et al., 2018. Immunotoxic and hepatotoxic effects of perfluoro-n-decanoic acid (PFDA) on female Harlan Sprague–Dawley rats and B6C3F1/N mice when administered by oral gavage for 28 days. Journal of Immunotoxicology, 15(1), 41–52. https://doi.org/10.1080/1547691X.2018.1445145	Immunotoxicity in rat, slight results incl. reduced phagocytosis.	2	One additional *in vivo* study seems available (PNDT).
PFBS 0.0085 mg/kg bw/d NOAEL = 50 mg/kg bw/d; **AF = 5875** (587.5 (interspecies: 235 (TK) x 2.5 (TD)) x 10 (intraspecies))	PNDT (GD1-20) oral gavage mice study; Feng et al., 2017. Exposure of Pregnant Mice to Perfluorobutanesulfonate causes Hypothyroxinemia and Developmental Abnormalities in Female Offspring. Toxicological Sciences, 155(2), 409–419. https://doi.org/10.1093/toxsci/kfw219	Changes in thyroid hormone	2	Several new animal studies are available: 28-day study rats (NTP, 2022); 90-day study rats (Lieder, Chang, et al., 2009); 4-6-weeks (one dose evel) male mice (Bijland et al., 2011); 2-gen study rats (Lieder, York, et al., 2009)).
PFHpS -	No animal studies available. New human data became available but no POD.	-	-	-
EFSA-4 (PFOA, PFNA, PFHxS and PFOS) Further assessment and Modelling needed	Several epidemiological studies were identified that could potentially change the TWI developed by EFSA (studies that (1) reported a dose-response association for an adverse effect and (2) the adverse effect was observed at PFOA concentrations below approximately 30 ng/ml). Further modelling similar to the EFSA approach would be necessary to check how these study results might influence the TWI developed by EFSA.	-	-	*(Shearer et al., 2021)* *(Souza et al., 2020)* *(Spratlen et al., 2020)* *(Liao et al., 2020)* *(Chang et al., 2022)* *(Jensen et al., 2022)* *(Zeeshan et al., 2021)* *(T.-W. Lin et al., 2020)*

Studies identified by UBA (2023)				
RfD **PoD / AF**	**Study**	**Toxicological effect**	**Klimisch**	**Overall database and remarks**
PFBA Gao et al. 2019; > 0.132 ng/ml serum UBA 2016 assessment: 3 µg/kg bw/d NOAEL = 6 mg/kg bw/d; **AF = 400** (2 (time) x 20 (interspecies: 8 (TK) x 2.5 (TD)) x 10 (intraspecies))	Gao et al., 2019: Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFASs) and Association between the Placental Transfer Efficiencies and Dissociation Constant of Serum Proteins–PFAS Complexes. UBA 2016 assessment: The critical study in the derivation of the existing drinking water limit for PFBA was a subchronic 90-day oral gavage study (J. L. Butenhoff, Bjork, et al., 2012). A NOAEL of 6 mg/kg bw/day.	- Increased incidence of follicular hyperplasia/ hypertrophy of the thyroid gland and hepatocellular hypertrophy in a 90- day study in male rats.	-	According to UBA, PFBA epidemiological data on developmental endpoints need further evaluation. The PoD from Gao et al. epidemiological study cannot be considered reliable until after the body of epidemiological studies on PFBA exposure and birth outcomes is synthesized and considered in the context of weight-of-evidence analysis.
PFPeA -	No relevant new animal studies or human studies available for PFPeA	-	-	-
PFHxA NOAEL = 15 mg/kg bw/d **AF = 8163** (817.5 (interspecies: 327 (TK) x 2.5 (TD)) x 10 (intraspecies)) Alternatively, NOAEL = 30 mg/kg bw/day	UBA 2016 assessment: The critical study in the derivation of the drinking water limit for PFHxA was a chronic oral study by Klaunig et al., 2015: Evaluation of the chronic toxicity and carcinogenicity of perfluorohexanoic acid (PFHxA) in Sprague-Dawley rats. Toxicologic Pathology, 43(2), 209–220. https://doi.org/10.1177/0192623314530532	Low urine pH values (may not be considered adverse); Alternatively NOAEL of 30 mg/kg bw/d based on nephro- and hepatotoxicity in female rats can be used)		Newer epidemiological studies were evaluated by UBA, however, there was a lack of adequate dose-response data suitable for identifying a point of departure to derive a drinking water limit for PFHxA.
PFHpA BMDL05 = 6.63 mg/kg bw/d; BMDL05 with PROCAST, NOAEL = 0.5 mg/kg bw/d; BMDL10: 8.32 mg/kg bw/d **AF = 16300-144200** (2 (time) x 815-7210 (interspecies: 326-2884 (TK based on half-live ranges) x 2.5 (TD)) x 10 (intraspecies))	ECHA registration dossier, study assessed by RAC; OECD TG 408 & 422 (90-d/reproscreen) in CD1 mice from 2017 (author is not named. study summary available), https://echa.europa.eu/documents/10162/c9c9b080-2704-3052-08a8-e1bb7964b1b8	Necrotic effects in the liver	1	One further *in vivo* study was identified by UBA, however only subacute (21 d) scenario (Li et al.). Epidemiological studies that evaluated exposure-response relationships in relation to untransformed measures of PFHpA do not have data that clearly demonstrate an exposure-response relationship exists with an adverse health outcome.

Studies identified by UBA (2023)				
RfD **PoD / AF**	**Study**	**Toxicological effect**	**Klimisch**	**Overall database and remarks**
PFUnDA NOAEL = 0.1 mg/kg bw/d **AF = 12600** (6 (time) x 210 (interspecies: 84 (TK) x 2.5 (TD)) x 10 (intraspecies))	OECD test guideline 422 (reproscreen) (Takahashi et al., 2014). PFUnDA administered by gavage to Crl:CD(SD) rats at 0 (vehicle: corn oil), 0.1, 0.3 or 1.0 mg/kg bw/day.	Liver toxicity in rats	1	Two further *in vivo* studies identified (Yang et al., 2021; Xin et al., 2022), no long-term duration studies available.
PFDoDA NOAEL = 0.1 mg/kg bw/d **AF = 12600** (6 (time) x 210 (interspecies: 84 (TK) x 2.5 (TD)) x 10 (intraspecies))	Kato et al., 2015 (2015). Repeated dose and reproductive/ developmental toxicity (OECD422) of perfluorododecanoic acid in rats. Environmental Toxicology, 30(11), 1244–1263. https://doi.org/10.1002/tox.21996	Liver weight in rats	1	Five further *in vivo* studies have been identified. UBA report does not explain why the lower PoD (Ding et al) is not proposed. Despite availability of several *in vivo* studies, none of them employed chronic exposure scenario.
PFNS -	No animal studies available. New human data became available but no POD.	-	-	-

Only for very few compounds that were included in the UBA literature review report (2023), subchronic or chronic toxicological data are available. Substances for which animal data were identified and proposed as PoD include PFDA, PFBS, PFHxA, PFHpA, PFDoDA, and PFUnDA. Due to interspecies differences, especially in the toxicokinetics, furthermore mainly short- and subchronic exposure duration studies, enormous high assessment factors were proposed for extrapolation to humans (see above table, overall AF range 400 to 144 200). The uncertainties with using these animal derived PoD are thus considered large. For five substances a review of the epidemiological data was recommended, including PFOA, PFOS, PFNA, PFHxS (i.e. the EFSA-4) and PFBA. For three substances in the PARC Aligned studies scope, no relevant data were identified (PFHpS, PFPeA and PFNS). Eight substances of the PARC Aligned studies scope were not included in the review by UBA.

The working group summarized as well the epidemiological evidence that has been identified by UBA (2023). The following table provides an overview of studies identified that are suggested to potentially change the EFSA TDI. The working group assessed the quality of each study identified in terms of overall confidence, risk of bias, and sensitivity. This assessment was carried out by one epidemiologist, but the aim is for an assessment by two independent epidemiologists specialising in environment and health.

For this evaluation, the studies, based on the published articles, were assessed across seven established domains: participant selection, exposure measurement, outcome measurement, potential confounding, analysis, study sensitivity, and selective reporting. This framework was acquired from the IRIS program, which adapted the Risk of Bias in Nonrandomized Studies of Interventions (ROBINS-I) tool (https://methods.cochrane.org/methods-cochrane/robins-i-tool) for use with the types of studies commonly encountered by the EPA (U.S. EPA, 2024). Each domain was assigned a score of "Good," "Adequate," "Deficient," "Not Reported," or "Critically Deficient," with rationales provided for the assigned scores. Once all domains were evaluated, a confidence rating of "High," "Medium," "Low," or "Uninformative" was assigned.

The primary concerns were identified as potential bias (factors not accounted for in the analyses that may affect the magnitude or direction of an effect) and sensitivity (factors that limit the study's ability to detect a true effect). For more information on the questions and considerations used to assess study quality in relation to each domain, refer to Appendix A of the EPA (2024).

Table XII.4: Human epidemiological studies identified by UBA 2023 and evaluated by the working group.

Overview of the human studies identified by EFSA as the point of departure (POD) for the derivation of the TWI as a HBGV									
Publication	**Study design**	**Health system**	**Participant Selection**	**Exposure and outcome measurement**	**Confounding**	**Analysis**	**Selective reporting**	**Study sensitivity**	**Overall confidence**
Grandjean et al. (2012)	Cohort	Immune system	The study included 656 singleton births, with 464 children (71%) followed up at age 7. Participants and nonparticipants did not differ. Immune-specific exclusions were applied.	The accuracy and reliability of the data was ensured by including, in each analytical series, quality control serum samples, calibration standards, and reagent and serum blanks. PCBs were also measured. Health outcomes were based on laboratory assessment, lack of blinding is not a concern.	The study has limited ability to differentiate effects for any individual PFAS from the other PFAS. Confounders were likely assessed based on data from medical records. No SES/seasonality.	PFAS and antibody concentrations were log transformed due to skew. No information was provided on the limit of detection (or method for handling non-detects). The study authors did not indicate any missing data. Linear regression was used.	No concern	Adequate sample size. Exposure distributions : PFOA (GM [25th-75th]: 3.20 ng/mL [2.56-4.01]) PFOS (27.3 ng/mL [23.2-33.1]).	**Medium confidence.** Large prospective study with minimal concern regarding reporting of LOD for exposure levels. Exposure distributions suggest the detection rate was not a concern.
Abraham et al. (2020)	Cross-sectional	Immune system (main outcome) Secondary outcomes: Cardio-metabolic	Study on 101 1-year-old children in Germany with data collected in late 1990s. Despite limited information on the sampling frame and participation rates,	PFAS concentrations were measured retrospectively in 2018 (samples were frozen at -80 °C). Large disparity between	Risk of residual confounding impacting results for the main outcome is moderate. No control for potential confounding for the secondary outcomes.	Analysis of the main outcome: Spearman correlation coefficients were used to identify relevant associations. A linear model adjusted for confounders was applied to antibody concentrations, with log-transformation of	No concern	Low sample size. The variations in PFOA/PFOS were adequate despite concerns for their	**Low confidence**. Concern for residual confounding; Results of the regression models only presented graphically. For the secondary outcomes, only

		outcomes Thyroid function Liver function	selection bias is judged to be small. Families with history of metabolic diseases could be more prone to volunteer.	PFOA/PFOS levels in breastfed and formula-fed children. Outcome determination was adequate except for previous infections (no use of doctor diagnosis criteria) and TG (fasting was not required).		skewed data. PFAS concentrations were divided into quintiles to identify differences. A breakpoint was estimated via piecewise linear regression for the highest dose. For secondary outcomes, Spearman correlation coefficients showed the direction of associations with outcomes but not their magnitude.		deviation during long-term storage.	correlation analyses. Conclusion based on the publication and supplementary material.
Overview of the human studies identified by UBA 2023, which could potentially change the EFSA – TWI.									
Publication	**Study design**	**Health system**	**Participant Selection**	**Exposure and outcome measurement**	**Confounding**	**Analysis**	**Selective reporting**	**Study sensitivity**	**Overall confidence**
Shearer et al. (2021)*	Nested case-control	Cancer	Good sample size. In this trial among all participants in the screening arm with available pre-diagnostic serum samples, there were 324 incident cases of renal cell carcinoma (RCC). Good matching with the controls.	Samples were analyzed in the same batch, with lab staff blinded to case/control status and quality control replicates included. RCC was identified by routinary cancer screenings.	Strategy for selecting potential confounders not explicitly identified, but selected adjustment factors seem appropriate with no key confounders missing.	Statistical analyses were appropriate. Correlations among PFAS were reported as Spearman correlation coefficients. Multivariable conditional logistic regressions were used for the main analysis.	No concerns	No concerns. Multiple sensitivity analyses performed.	**Medium to high confidence.** No accounting of SES and lack of details on confounder selection. Strength is the study design.

Chang et al. (2022)	Cohort	Fetal growth and birth outcomes	This prospective cohort study enrolled African American pregnant women. The study provided clear sampling criteria but lacked information on the participation rate or non-participants, making it difficult to fully assess selection bias despite comparing the sample to the US Census survey for generalisability.	Analytical methods seem sound and sensitive with reasonable quality assurance data presented including inter-laboratory differences. Early sampling in trimester one and two is assumed to reflective of levels in critical windows (given the long half-lives of PFOS and PFOA) with minimal non-differential measurement error anticipated. Birth weight obtained from medical records, GA obtained from ultrasound data. The lack of race-specific values for SGA classification may lead to misclassification and reduced	The study did not explore different functional forms for some covariates, potentially leaving residual confounding, but the primary approach to confounding, guided by a relevant DAG, appears reasonable, though the impact of other PFAS and pregnancy hemodynamics remains unclear, with early sampling likely minimizing the latter.	Analyses were rigorous, detailed and appropriate for the study design and available data.	No concern	Higher than expected preterm birth and SGA prevalence (birth defects comparable at 3%), so could be indicative of higher at-risk population.	Minimal bias concerns for birth weight but was underpowered for most PFAS and had potential misclassification for SGA, lowering confidence in that outcome. **Medium confidence** for birth weight, **low confidence** for SGA.

				sensitivity in detecting true SGA cases.					
Jensen et al. (2022)	Cross-sectional	Thyroid function	The study population was representative of the cohort. However, no comparison participants vs. non-participants. 1048 pregnant women were included.	QA/QC measures were robust. Timing of exposure measurement (first trimester) was appropriate for assessing thyroid hormone effects.	Adjustments by key confounders. No adjustment by iodine status.	Multivariate linear regression methods were used. Sensitivity analyses were conducted.	No concern	The study had an adequate sample size and measured PFAS and thyroid hormones in the same laboratory, reducing variability. However, the cross-sectional design limits causal inference, and the exposure range was relatively narrow, which could reduce sensitivity to detect associations.	**Medium confidence**. Potential residual confounding and cross-sectional design

T-W Lin et al. (2020)	Cross-sectional	Cardio-metabolic outcomes	Although the recruitment was clear and based on established criteria, the relatively low participation rate (42.3%) and potential healthy participant bias could have influenced the results.	The approach to measuring PFAS concentrations was detailed. Results related to fasting glucose, triglycerides, and uric acid could have shown variability due to confounders like lipid-lowering drugs, which may have altered the PFAS-lipid relationship.	Adjustments by key confounders. No adjustment by other potential confounders (diet, exercise).	The statistical analyses was appropriate. Linear regression models were used.	No concern	The lack of clear handling of missing data and some decisions on variable exclusions may have reduced the sensitivity of the study	**Medium confidence**. The study has limitations in participant selection, residual confounding, and lack of handling of certain factors may reduce confidence in the findings.
Souza et al. (2020)	Case-control	Fetal growth and birth outcomes	The recruitment and sampling were well-detailed, though non-participant comparisons were missing; random selection of controls and minimal follow-up loss suggest low selection bias potential.	Standard methods were used for sampling and storage, with sensitive PFAS analysis in the second trimester, aligning with critical exposure windows due to their long half-lives. Intrauterine growth retardation was estimated by Fetal Growth	Most key confounders were adjusted for, but some decisions, like adjusting for preterm birth (a potential causal intermediate), were questionable.	Analyses were basic but appropriate for a case-control study, though some details on methods were missing	No major concerns, although it remains unclear whether other outcomes were examined and just not reported.	Median (IQR); min, max for 252 samples PFOS: 3.41 (3.43); 1.06, 106) ng/mL; Detection rate: 100% PFOA: 0.20 (0.22); 0.11, 2.77) ng/mL; Detection rate: 68% Good quantile exposure contrasts for PFOS; limited contrasts for	**Low confidence**. Strengths include a nested case-control design, but limited details and whole blood use may reduce sensitivity and increase uncertainty.

				Ratio. No reporting of gestational age dating techniques. No criteria reported for preeclampsia.				PFOA.	
Spratlen et al. (2020)	Cross-sectional	Cardio-metabolic outcomes	The study population was drawn from a well-defined cohort of pregnant women exposed to the World Trade Center disaster. Inclusion/exclusion criteria were clearly described. However, participation rates were not explicitly discussed.	PFAS levels were measured in cord blood using validated methods. The analytical approach was robust, with high recovery rates and low limits of quantification. Lipid levels were measured using standard enzymatic methods.	The study adjusted for key confounders (e.g., maternal age, race, BMI, education, smoking status, gestational age). The rationale for confounder selection was based on prior literature.	The analysis used linear regression models, adjusted for confounders, and included sensitivity analyses. Missing data were handled appropriately (imputation for values below LOQ).	No concern	The study had a sufficient sample size (N=222) and a wide range of PFAS exposure levels. The population was relevant to the research question, and the exposure range was adequate.	**High confidence.** The study is well-designed, with robust methods for exposure and outcome measurement, adequate control of confounding, and appropriate statistical analysis. Limitations are minor and unlikely to affect results. The primary weakness is the cross-sectional design.
Zeeshan et al. (2021)	Cross-sectional	Cardio-metabolic outcomes	The study is a cross-sectional investigation with a large sample size (n=1045). Participants were recruited from Shenyang, China, with clear inclusion/exclusion criteria	Single-time measurement may not capture long-term exposure variability. T2D was defined using fasting plasma glucose (>7.0 mmol/L), a standard clinical criterion.	The study adjusted for multiple confounders (age, sex, ethnicity, diet, income, smoking, etc.) using directed acyclic graphs (DAGs) to identify optimal covariates. Sensitivity analyses were also performed.	Multivariable linear and logistic regression models were used. PFAS concentrations were log-transformed, and quartile analyses were performed. Missing data handling and sensitivity analyses were described.	No concern	The study had a large sample size and high detection rates for PFAS. However, the cross-sectional design limits the ability to establish	**Medium confidence.** The study is well-conducted with robust methods, but the cross-sectional design and single-time exposure measurement limit causal inference and introduce potential for non-differential misclassification.

				Glucose-homeostasis markers (e.g., HOMA-IR) were also measured using validated methods.				temporal relationships.	
Liao et al. (2020)	Cross-sectional	Cardio-metabolic outcomes	Data from NHANES. Loss to follow up is not a concern due to cross-sectional design. Inclusion criteria for the present study were not likely to introduce selection bias.	QA/QC procedures are provided. PFOA and PFOS were detected in >98% of samples, and values below the LOD were recorded as the LOD divided by the square root of 2. Outcome measures are good.	A priori selection of confounders/covariates. Adjustments of the models done by sets.	Multivariable logistic regression models were used for the main analysis. They included piecewise models used to address nonlinear relationships.	No concern	The sample size was large. PFOS had the largest interquartile range (Table 1). Temporally, the exposure and outcome were measured within the same NHANES sampling cycle.	**High confidence.** The primary weakness is the cross-sectional design.

Overview of other relevant human studies mentioned in the UBA report									
Publication	**Study design**	**Health system**	**Participant Selection**	**Exposure and outcome measurement**	**Confounding**	**Analysis**	**Selective reporting**	**Study sensitivity**	**Overall confidence**
Grandjean et al. (2020)	Cross-sectional	Immune system	Use of the national registry data, no concerns for selection bias. Some concern for representativeness of the target population.	Exposure timing is appropriate as COVID is acute, and current immune function is relevant. Due to the use of national registry data, any outcome misclassification is not expected to vary with exposure status.	Potential confounding variables were identified based on a priori knowledge.	For the main analysis, logistic regression models were used. Missing data are not addressed.	No concern	Adequate sample size and exposure contrast. No other concerns for reduced sensitivity.	**Medium confidence.** No bias in exposure or outcome measurement, but the group may represent more severe COVID-19 cases or heightened concern
Grandjean et al. (2017a)	Cohort and cross-sectional	Immune system	Repeated measurements. (18 months and 5 years) No differences with non-participants.	PFHxS, PFOA, PFOS, PFNA, and PFDA. The accuracy and reliability of the data was ensured by including, in each analytical series, quality control serum samples, calibration standards, and reagent and serum blanks. Within-batch and between-	Basic adjustments from medical records. There is no information on validity of data from medical records. Because of the close correlation of the various PFASs, meaningful adjustment for concomitant PFAS exposures could not be conducted.	Statistical analyses were appropriate. Results were reported for cohort-specific and pooled analyses. No evidence of missing outcome data.	No concern	Adequate sample size. Median (25th -75th percentiles ng/mL) maternal: 1.0 (0.6-1.5); age 5: 1.1 (0.8-1.6)	**Medium confidence**. Identified limitations unlikely to be severe.

				batch imprecision levels (coefficients of variation) for all five PFASs were <3% and 5–6%, respectively. Timing of exposure measurement is appropriate. Antibody measures were analyzed in a separate laboratory, therefore, blinding is not a concern.					
Grandjean et al. (2017b)	Cohort and cross-sectional	Immune system	Participant follow up from Grandjean 2012. No differences with non-participants.	The accuracy and reliability of the data was ensured by including, in each analytical series, quality control serum samples, calibration standards, and reagent and serum blanks. PCBs were also measured. Antibody measures for IgG were	Adjusted by PCBs and other basic confounders from medical records. Key confounders not considered included SES and season of outcome measure.	Statistical analyses were appropriate. There is little missing data and all were appropriately addressed	No concern	Adequate sample size, but exposure levels are low with narrow contrast. Mean (25th - 75th percentiles) (ng/mL): Maternal: 0.6 (0.5-0.8) Age 5: 1.0 (0.8-1.2) Age 7: 1.1 (0.9-1.5)	**High confidence**. It was a well-conducted study with minimal concern for bias.

				performed in a separate lab from the exposure assessment; therefore, blinding not a concern for these endpoints.					
Gao et al. (2019)	Cross-sectional	Fetal growth and birth outcomes	Recruitment of pregnant women from 2015 to 2016. Low evidence for selection bias.	They followed rigourous QA/QC procedures. Information of the outcomes obtained through medical records. For gestational age, dating information was not provided.	Statistical criteria using p-values in univariate models were only for identifying confounders. Overfitting due to highly correlated variables (e.g., BMI, height, weight) is evident, though its impact on precision is unclear. Some important confounders, like SES markers, are missing.	Decisions to analyse data based on very small sample sizes (n=132) is questionable. Stratified and sensitivity analyses were not considered and/or reported.	No concern	Median; Mean (Min-Max) ng/mL; % BLOD PFOS Maternal Serum: 4.07, 4.64 (0.07 to 22.6); 0 PFOS Cord Blood: 1.8, 2.35 (0.04 to 8.01); 0 PFOA Maternal Serum: 2.21, 2.85 (BLOD to 25.4); 6% PFOA Cord Blood: 1.88, 2.29 (0.03 to 10.2); 4% PFHxS Maternal Serum: 0.24, 0.28 (BLOD-1.15); 9% PFHxS Cord	**Low confidence**. While the study had some strengths, significant deficiencies in confounding and analysis contribute to uncertainty.

								Blood: 0.11, 0.17 (0.05-0.83); 28% PFNA Cord Blood: 0.38; 0.51 (0.05-2.9); 1% PFNA Maternal Serum: 0.57, 0.69 (BLOD - 3.98); 2% PFDA Maternal Serum: 0.47, 0.57 (BLOD - 3.15); 5% PFDA Cord Blood: 0.21, 0.26 (0.03-1.11); 7%	
Budtz-Jørgensen & Grandjean (2018)	Cohort	Immune system	The study was conducted in a population with a high participation rate and homogeneity, which strengthens the selection process. Findings might not be generalizable to other populations.	Exposure were analysed using reliable biomarkers. Outcomes were measured with standard and validated methods.	While major confounders like sex and age were controlled for, there could still be residual confounding due to co-exposure to multiple PFASs.	The analysis used benchmark dose (BMD) methodology, including linear and piecewise linear models, and adjusted for co-exposures. Sensitivity analyses were conducted to assess model robustness.	No concern	Adequate sample size. No concerns.	**High confidence.** Well conducted study with minimal concern for bias. linear dose-response models. Mixtures assessment.

Averina et al. (2021)	Cross-sectional	Cardio-metabolic outcomes	2010-2011. Although there is no comparison of those who did and did not participate at baseline or for provision of blood samples, the study design and high participation rates lessen concern for selection bias. Healthy participants.	PFAS values below the LOD were replaced with LOD/2. Main outcomes: cholesterol, HDL, LDL, dyslipidemia, hypertension, and obesity. Some little concern for reverse causality due to lipid binding by PFAS (people with a metabolic disease might have more accumulation of PFAS, not because PFAS caused the disease, but because the bodies tend to accumulate more fat). No mention of fasting.	Models included age, sex, BMI, physical activity, use of chewed tobacco, and dietary variables. Selection of confounders *a priori*.	Associations between PFAS and lipid outcomes were evaluated using linear or logistic regression. A principal components analysis was also conducted to evaluate mixture effects of co-occurring PFAS. Missing data approach is not described.	No concern	Adequate sample size (n=940). Adequate exposure contrast for PFOS/PFOA. PFOS median (IQR) in boys: 6.52 ng/mL (3.09 ng/mL); PFOS median (IQR) in girls: 5.71 ng/mL (2.64 ng/mL); PFOA median (IQR) in boys: 1.86 ng/mL (0.67 ng/mL); PFOA median (IQR) in girls: 2.14 ng/mL (1.26 ng/mL)	**Medium to low confidence.** cross-sectional study, no evidence of significant bias in selection or exposure. There is potential concern for outcome misclassification due to triglycerides being an outcome without accounting for fasting. However, time since last meal was adjusted for in statistical models. Potential reverse causality due to metabolic changes in obese individuals.
Wikström et al., (2020)	Cohort	Fetal growth and birth outcomes	2355 pregant women. Longitudinal pregnancy cohort. Based on the description of recruitment process, it is not	QA/QC reporting in sister paper was appropriate and indicative of accurate measures. Outcome	Approach for assessing confounding: Approach: Use of R-squared statistics for stepwise regression model fit to determine which covariates were included in the final	Single multiple linear and logistic regression models based on PFAS exposure quartiles and a one ln-unit change. Multicollinearity was evaluated. Sensitivity analyses were conducted.	No concerns	Study appeared to have sufficient power to detect sex-specific PFAS results based	**High confidence**. This was a prospective cohort study with many study strengths including sufficient study sensitivity and largely sound methodological

			anticipated that any self-selection factors of the underling participants or those providing blood samples are related to both the underlying PFAS exposure and the eventual outcomes; thus, there is minimal concern for selection bias.	measurement error and misclassification is expected to be minimal for these well-characterized measures.	modelling.			on SDS birthweight, suggests that the exposure contrast was also sufficient; however, marginally statistically significant interactions (p-values of 0.06 and 0.07 for PFOA and PFOS) for relatively large (upwards of 100-gram) sex-specific differences which suggest power to detect interactions may be limited.	approaches and design, as well as no clear evidence of Bias.

***Carcinogenic endpoint. EPA selected the critical effect of renal cell carcinoma in men reported by Shearer et al. (2021) as the basis of the overall CSF for PFOA.**

In summary of table XII.4, epidemiological human studies reported by UBA tend to have medium confidence in terms of results reporting, risk of bias, and study sensitivity. There are 5 studies classified as they are of high confidence (30%) and 4 studies classified as low (23%). The study evaluation was based on the evaluation of the different domains included in the table: study design, participant selection, exposure and outcome measurement, confounding, statistical analysis, selective reporting, and study sensitivity. High Confidence studies have the highest evaluation ('Good') for all or most domains; Low Confidence studies have a 'Poor' evaluation for one or more domains (unless the impact of the particular limitation(s) is judged to be unlikely to be severe), and Medium Confidence studies are in between these groups (e.g., most domains receiving a mid-level Adequate evaluation, with no limitations judged to be severe). In general, the studies had cross-sectional designs, limited approach to assess potential confounders of the associations, and very specific populations (which make the conclusions difficult to be generalized to the whole population). After this evaluation of the studies reported by UBA, the working group decided to investigate the meta-analyses published in the scientific literature on PFAS and health outcomes, to further investigate which health endpoint is more generalizable and critical to PFAS's effects (see chapter 5).

4.3 Summary and conclusions on available assessments (non-cancer)

From the above tables XII.1 and XII.2 summarizing available health-based HBM-GV and reference doses based on human and animal data for non-cancer effects, the following observation can be summarized:

The assessment of the German Biomonitoring Commission is the only primary assessment that aimed in deriving Human Biomonitoring Guidance Values. Most agencies aimed in deriving reference values in term of a delivered dose, such as TDI or TWI, MRL, or RfD, that serve a different purpose (e.g. deriving precautionary drinking water limits) than assessing measured HBM serum levels of a population regarding the likelihood of being associated with certain health impacts.

Until today, most of the assessment focused on PFOS and PFOA.

Values were derived based on human data, where such evidence was available (e.g. US EPA, CalEPA, EFSA, German HBM), but some agencies derived assessment values based on animal data, either because reliable human data are lacking for a specific compound or animal data were preferred.

The assessment of EFSA (2020) is until today the only primary assessment deriving a guidance value for a mixture of PFAS. The assessment did not aim in deriving an HBM-GV but rather a TWI expressed as delivered dose. Still, it may be concluded that the corresponding serum concentration is regarded as acceptable level even if not explicitly stated.

It overall appears that the assessments of point of departures for PFOS, PFOA and EFSA-4 conducted earlier (German HBM Commission 2015/2016; 2021; EFSA, 2020) and more recently (US EPA, 2024) appear broadly in line regarding the non-carcinogenic effect domains and critical effect levels despite further evolution of knowledge. The most recent assessment, by U.S. EPA, identified for PFOA the immunotoxicity, developmental toxicity and metabolic effects (cholesterol) as co-critical effects for PFOA. The EFSA-4 is based on immunotoxicity as critical effect, however meant to be protective also for other effects. Critical serum levels for PFOA are broadly in line (German HBM-I: 2 ng/mL; EFSA (PFOA+PFNA): 2 ng/mL; U.S. EPA: Immunological effects (↓serum IgG): 1.24-3.47 ng/mL)).

Diverging opinions have however been developed concerning PFOS and PFOA effects on serum cholesterol. While EFSA, in 2018, considered the increase of serum cholesterol to be the critical key adverse outcome for PFOA and PFOS, this judgement has been revised in its 2020 opinion, considering the uncertainty due to reverse causality of the association between increased PFAS serum levels and increased total cholesterol as too high. In contrast, Californian EPA (OEHAA 2024) presents a detailed assessment concluding in summary that the current epidemiologic literature provides evidence that PFOA and PFOS can cause increased total cholesterol in humans. Also, the U.S. EPA (2024) considers PFOA and PFOS effects on serum cholesterol as co-critical PoD for RfD derivation. Finally, in the recently published IARC Monograph on PFOA and PFOS it is noted by the IARC Working Group "that the existing evidence does not support the proposed mechanisms of reverse causality between the measured PFAS and serum cholesterol" (IARC 2025).

The institutions and experts derived guidance values ultimately differ. RfDs derived by U.S. EPA (2024) and Tewlik (2023, based on U.S. EPA) are lowest, attributable to an additional assessment factor of 10 taken into consideration for general population variability. In contrast to EFSA and the German HBM Commission, where no additional assessment factors have been applied to the critical serum levels identified in humans.

Especially the values on additional individual PFAS (beyond PFOS, PFOA, EFSA-4) were proposed mainly based on animal data. Where animal data were used (e.g. ATSDR, Dr. Voss on behalf of UBA, US EPA) the low confidence and uncertainties associated with the point of departures and extrapolations were highlighted, including issues such as only subacute studies were available, lack of studies that specifically evaluated health effect domains of emerging concern across the PFAS class, large interspecies differences in toxicokinetics (elimination half-lives), or only administered doses but no serum concentrations were measured. The uncertainties were addressed in different ways: applying large assessment factors, values were labelled "provisionally" (Dr. Voss on behalf of UBA) or no values were derived at all for the chronic exposure scenario (ATSDR, HBM4EU).

Overall, it needs to be concluded that animal derived guidance values are of low confidence.

Overall, considering the uncertainties related to data-scarcity and interspecies differences the working group has reservations to use animal data to derive individual HBM-GV for the sum-value approach or for the RPF approach, respectively.

In general, the working group shares the view of expert agencies such as EFSA and ATSDR that RPF is uncertain to apply to PFAS.

Liver effects may not be considered the relevant endpoint for humans. There is uncertainty on potential multiple mechanisms for liver effects, among which peroxisome proliferation is a likely contributor, a mechanism however which may not be reliably extrapolated to humans (Hall et al. 2012). In line ATSDR (2021) considered increases in liver weight and hepatic lipid levels and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, not appropriate endpoints for deriving MRLs. The working group notes an additional publication (Bil et al., 2023b) is available, where internal RPFs were derived for PFAS based on rat thymus weight, spleen weight, and globulin concentration. Some uncertainties in this approach are noted by the working group, including the use of short duration (subacute) studies and using absolute lymphoid organ weights as endpoint. No sensitive functional endpoints, nor histopathological correlates were considered. Considering furthermore internal PFAS levels were not measured in the animal studies and PK modelling was employed to obtain internal from external RPFs, finally extrapolation of these RPFs to humans, add further layers of uncertainty. While higher potency was derived for PFOS than for the reference substance PFOA, and very distinct potencies for PFNA (higher than PFOS) and PFHxS (low), of note are further recent findings from *in vitro* and in *silico* studies on immunotoxicity indicating that PFOA and PFOS were similarly potent and more potent than PFNA and PFHxS, which exhibited similar potency to each other (Corsini et al 2024).

The overall uncertainties thus may be high and the relevance of these RPF based on immune-related endpoints for humans is unknown.

Overall, data suggest that the toxicity of these substances seem mediated by multiple receptors, including PPARα, CAR, and PXR, and that there may be species differences in the response mediated by different receptors. Additionally, available data suggest there may be qualitative differences in the toxicities of various PFAS, introducing large uncertainties in the RPF approach. In particular, concerning PFOA, when used as reference substances, it is stressed that it may exhibit very distinct effects and potency, such as carcinogenicity (see section below).

Worth however to mention that it might be expected that different perfluorinated compounds induce as combined exposures several key events in few Adverse Outcome Pathways. To identify and assess these combined exposures it might be beneficial to add effect-biomonitoring approaches for example PFOA receptor assays as integrative measures and to define health based-mixture thresholds according to OECD guiding principles (OECD 2025 in prep.).

4.4 Carcinogenicity

An important evolution in the PFAS assessment since 2020 is the carcinogenicity classification by IARC and assessment of carcinogenicity of PFOS and PFOA by U.S. authorities EPA and CalEPA.

4.4.1 Cancer classifications

In 2024, IARC classified PFOA and PFOS for carcinogenicity. The IARC Monograph on PFOA and PFOS was published only recently (IARC, 2025).

Eligible studies considered by IARC were those published or accepted for publication in the openly available scientific literature by the time of the Working Group meeting, which was November 2023.

IARC classified PFOA as carcinogenic group 1 (carcinogenic to humans) based on "*sufficient evidence"* in animals (NTP, 2020), based on "*limited evidence*" in human for RCC and testicular tumors referring to their previous evaluation and to Shearer et al. (2021), furthermore based on "*strong*" mechanistic evidence.

IARC considered mechanistic evidence strong in exposed humans. PFOA was found to induce epigenetic alterations (gene-specific DNA methylation in children, persistence of PFOA-associated CpG-methylation between birth and adolescence, alterations in expression of cancer-related miRNAs).

PFOA was shown to be immunosuppressive because multiple well-conducted studies in different populations of exposed humans, incl. children and adults, have shown that exposure to PFOA is associated with increased risk of infectious disease and decrease vaccine response.

Furthermore, there is evidence on decreased cytokine production and reduced lympho-proliferation in human primary cells and by altered antibody responses to T-cell-dependent antigens in leukocytes of rodents. Furthermore, PFOA induces oxidative stress, modulated receptor-mediated effects, alters cell proliferation, cell death and nutrient supply. Mechanistic evidence is also supported by metabolomics and transcriptomic data.

IARC classified PFOS as carcinogenic group 2B (possibly carcinogenic to humans) based on "*strong*" mechanistic evidence and "*limited* evidence" in experimental animals. The evidence in humans was considered "*inadequate*".

Animal evidence was concluded based on Butenhoff et al., (2012), reporting hepatocellular adenoma and hepatocellular adenoma and carcinoma combined with positive trend in female rats, thus the neoplastic finding is limited to one sex of a single species in a single study and combining benign and malignant tumors. In this study, the liver adenoma incidence had not a clear dose-response in males (0/60, 3/50, 3/50, 1/50, 7/60* for 0, 0.5, 2, 5, 20 ppm, respectively; * stat. sign p<0.05), and fully recovered in the recovery group with 0/40 animals. In females, incidences were 0/60, 1/50, 1/49, 1/50 and 5/50* for 0, 0.5, 2, 5, 20 ppm (*stat. sign p<0.05), respectively; with one carcinoma in the high dose, thus the neoplastic response was limited to the high dose and this 20 ppm group females resulted in a statistically significant increase in combined hepatocellular adenoma and carcinoma.

Evidence in humans was inadequate because in the few available studies, positive findings appeared sporadically and inconsistently for a few cancer sites (testis, breast, thyroid).

Two regulatory carcinogenicity assessments of PFOA and PFOS have been published in recent years by U.S. authorities.

U.S. EPA (2024) classified PFOA and PFOS according their classification scheme. The key studies referred to by IARC were considered by EPA (2024) in its PFOA cancer assessment. EPA determined PFOA is "*Likely to Be Carcinogenic to Human*". For PFOS, EPA reviewed the weight of the evidence and determined that PFOS is "*Likely to Be Carcinogenic to Humans*".

4.4.2 Published cancer risk assessments

U.S. EPA and Californian EPA (OEHAA, 2024) derived cancer slope factors based on the published animal and human data. The literature review of U.S. EPA considered studies until February 2023. The literature review of OEHAA considered data until September 2022. In addition, Steenland et al. (2022) published a cancer

risk assessment for PFOA using pooled individual level data from two epidemiological studies. The following table summarizes the assessments:

Table XII.5: Cancer risk estimates based on human and animal data

Cancer - Human data derived risk values (based on serum or blood levels)		
Compound/value	**PoD**	**Details**
U.S. EPA (2024) **PFOA** **$CSF_{serum\ (UCL)}$ = $3.52x10^{-3}$ $(ng/mL)^{-1}$** **$CSF_{serum\ (central)}$ = $1.78x10^{-3}$ $(ng/mL)^{-1}$**	**Cancer slope factor derivation based on human data.** Various candidate CSF based on human and animal data were derived. The overall CSF supported by EPA for PFOA was based on RCC in humans reported by Shearer et al. (2021): 0.0293 $(ng/kg/day)^{-1}$ (29,300 $(mg/kg/day)^{-1}$) or **$3.52x10^{-3}$ $(ng/mL)^{-1}$**. (The estimated CSF_{serum} of $3.52x10^{-3}$ (ng/mL)–is based on 95% CL of the dose-response slope (b); the CSF based on the estimated slope b (central estimate) is **0.00178 $(ng/mL)^{-1}$**.	Shearer et al., (2021) was considered as PoD for cancer risk assessment. The Shearer et al study population constituted of males and females aged 55-75 yrs. Note: CSF (absolute risk scale) were calculated by multiplying the lifetime risk of kidney cancer in U.S. males (American Cancer Society, 2020) by the percentage of all kidney cancers that are the RCC subtype (90%). This gave an R0 of 0.0202 × 90% = 0.0182. The CSF_{serum} was then calculated as the product of the upper 95% CL of the dose-response slope (b) and R0. EPA (2024): FINAL Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts.
CalEPA (2024) **PFOA** **CSF_{serum} =** **$1.78x10^{-3}$ $(ng/ml)^{-1}$ (Shearer)** **$2.9x10^{-4}$ $(ng/ml)^{-1}$ (Vieira)**	**Cancer slope factor derivation based on human data.** Linear regression for PFOA based on RCC in humans (Shearer et al., 2021 and Vieira et al, 2013). **CSFserum of 0.00178 $(ng/ml)^{-1}$ (Shearer) and 0.00029 $(ng/ml)^{-1}$ (Veira)**; or CSF_{intake} of 0.00637 $(ng/kg\ bw/d)^{-1}$ (Shearer) and 0.00105 $(ng/kg\ bw/d)^{-1}$ (Veira). The geometric mean of the CSF_{intake} values from the Vieira et al. (2013) and Shearer et al. (2021) studies is 0.0026 $(ng/kg\text{-}day)^{-1}$ of PFOA intake.	OEHHA considered only human data and selected the studies by Shearer et al. (2021) and Vieira et al. (2013) as PoD for cancer dose-response analyses, but Barry et al., 2013 and Steenland and Woskie (2012) were also compared. OEHHA 2024: Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water.
Steenland et al (2022) **PFOA** **CSF_{serum} = $1.79x10^{-3}$ $(ng/mL)^{-1}$**	**Cancer slope factor derivation based on human data.** Using pooled dataset from Shearer et al. (2021) and C8 Science Panel data (Barry et al. 2013). For CSF derivation, using the coefficient from the slope below the knot in the untransformed 2-piece linear spline model.	Steenland et al. (2022) pooled individual data from NCI study by Shearer et al. (2021) and C8 science panel study by Barry et al. (2013). Use of two-piece linear spline with linear slope below the knot at the 60th percentile of serum levels in the pooled data (and the 85th percentile of the NCI distribution) (approx. 10 ng/ml). C8 panel data were first adapted to combine with NCI data, constructing nested case-control risk sets.

Cancer - Human data derived risk values (based on serum or blood levels)		
Compound/value	**PoD**	**Details**
U.S. EPA (2024) **PFOS** **CSF = 39.5 (mg/kg bw/d)$^{-1}$**	**Cancer slope factor derivation based on animal data.** CSF of 39.5 (mg/kg/day)$^{-1}$ derived based hepatocellular adenomas and carcinomas in female rats reported by Butenhoff et al. (2012)/Thomford (2002b) for PFOS. PoD = BMDL10: $PoD_{Internal\ Dose\ Metrix}$: 19.8 mg/L ($AUC_{avg}$); PoD_{HED}: 2.53x10^{-3} mg/kg bw/d. CSF = BMR/PoD_{HED}).	Study details see above under cancer classification of PFOS by IARC. Tumorigenic dose was the highest dose tested of 20 ppm or approximately 1.25 mg/kg bw/d. EPA selected the AUC averaged over the study duration (AUC_{avg}), equivalent to the mean serum concentration over the duration of the study, as the dose metric for modeling cancer endpoints. BMDL10 derived by BMDS 3.2 program. The animal POD was converted to a PODHED by multiplying the POD by the human clearance value. EPA (2024): FINAL Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts.
CalEPA (2021) **PFOS** **CSF = 15.6 (mg/kg bw/d)$^{-1}$**	**Cancer slope factor derivation based on animal data.** CSF of 15.6 (mg/kg-day)$^{-1}$ based on the combined incidence of liver and pancreatic tumors in male rats reported by Butenhoff et al. (2012). PoD = BMDL05: PoDInternal Dose Metrix: 14.7 mg/L; PoD_{HED}: 3.2x10^{-3} mg/kg bw/d. CSF = BMR/PoD_{HED}).	CalEPA applies linear extrapolation because "*there is evidence to suggest that PFOS may be genotoxic and has multiple key characteristics of carcinogens. PFOS likely acts through multiple MOAs, and presently, the evidence does not support excluding genotoxicity as a possible MOA for carcinogenicity.*" OEHHA 2024: Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water.

PFOA cancer risk assessment

Concerning PFOA, in humans, U.S. EPA for its quantitative risk assessment considered results are most consistent for kidney cancer in adults based on a nested case-control study (Shearer et al., 2021), two C8 Health Project studies (Barry et al., 2013; Vieira et al., 2013), two occupational mortality studies (Raleigh et al., 2014; Steenland and Woskie, 2012), and a meta-analysis of epidemiological literature that concluded that there was an increased risk of kidney tumors correlated with increased PFOA serum concentrations (Bartell and Vieira, 2021). Therefore, the endpoint of kidney cancer was selected by U.S. EPA for CSF derivation. Among epidemiological studies examining risk of testicular cancer, two studies in the same high-exposure community (C8 Health Project) reported this association (Barry et al., 2013; Vieira et al., 2013). Overall, two studies, Shearer et al., and Vieira et al., were retained for CSF derivation by US EPA. Concerning animal data, according to EPA, three chronic studies are available that investigate the relationship between dietary PFOA exposure and carcinogenicity in male and female rats (NTP, 2020; Butenhoff et al., 2012; Biegel et al., 2001). Combined, at least two of the three studies report increased incidences each of hepatic, testicular, and pancreatic neoplastic lesions. Increased incidences of neoplastic lesions were primarily observed in male rats, though results in females, particularly the reports of rare tumor types (i.e., pancreatic acinar cell adenomas and adenocarcinomas), were considered supportive of potential carcinogenicity of PFOA. NTP (2020) was selected for candidate CSF derivation for the Pancreatic Acinar Cell Adenoma or Adenocarcinoma and the hepatocellular tumors; while Butenhoff et al. (2012) was selected for candidate CSF derivation for testicular Leydig Cell Adenomas.

In EPA`s overall evaluation, human data were preferred as it limits uncertainties associated with interspecies extrapolation and the toxicokinetic differences between species, regarded as major uncertainties associated

with the PFOA animal toxicological studies due to the half-life differences and sex-specific toxicokinetic differences in rodent species. The use of human data also ensures that the values are based on human-relevant exposure conditions and human-relevant tumor types/sites. EPA selected the critical effect of renal cell carcinomas in men reported by Shearer et al. (2021) as the basis of the overall CSF for PFOA.

Shearer et al. (2021) is a multicenter case-control epidemiological study nested within NCI's PLCO with median PFOA levels relevant to the general U.S. population (N= 326). Median PFOA levels in controls was 5.0 ng/mL, comparable with 4.8 ng/mL in adults aged 60 and over from NHANES 1999–2000. Exposure percentiles were 4.0, 5.6, and 7.3 ng/ml for 25th, 50th, and 75th percentile respectively. EPA considered this study of medium confidence. The NCI study was nested in a large cohort of approximately 150,000 participants with measured PFOA in stored pre–diagnostic serum samples that were followed for over 20 years, so there are few or no concerns regarding control selection, survivor bias, temporality, or other issues compromising validity. The study controlled for potential confounding by individually matching on age at enrollment, sex, race and ethnicity, study center, and study year of blood draw. One limitation notes is exposure classification based on a single blood sample drawn for each participant. According to Shearer et al, due to the relatively long half-life of PFOA and evidence from repeated measurements in another general population study (Sun et al. 2018) it was suggested that a single measurement can be a good proxy for a longer term PFOA exposure in the general population. The study reported a statistically significant increased risk of kidney cancer (RCC) associated with pre-diagnostic serum levels of PFOA when analysed categorically (OR = 2.63; 95% CI: 1.33, 5.20 for the highest vs. lowest quartiles; p-trend = 0.007), or continuous (i.e. per doubling of PFOA: OR: 1.71; 95% CI: 1.23, 2.37). When adjusting for other PFAS the association remained significant in analyses on a per doubling increase in PFOA. The increase in OR remained across the quartiles, the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS in the highest vs. lowest quartiles), however it was no longer significant when analysed categorically. Increased OR of RCC were specifically observed in participants` ages 55–59 years, and in men and in women, separately (see Appendix D, (U.S. EPA, 2024a)).

CalEPA concluded that the four human studies (Steenland and Woskie, 2012; Barry et al., 2013; Vieira et al., 2013; Shearer et al., 2021) have adequate data to evaluate an association between PFOA and kidney cancer and all reported strong evidence supporting a true causal association between PFOA and this cancer type. Evaluations of chance, bias, confounding, dose-response, consistency, and biologic plausibility supporting these findings. Of the four human studies the Vieira et al. (2013) and Shearer et al. (2021) studies were selected for dose-response assessment and cancer slope factor (CSF) calculations.

Neither U.S. EPA nor CalEPA in its cancer risk assessment impute a threshold, applying a linear extrapolation based on the ORs obtained in the categorical analysis of the data, where a non-zero referent exposure group, the low exposed group (25th exposure percentile), was considered.

Steenland et al. (2022) approached quantitative dose-response modelling with a pooled analysis of individual-level data of the studies published by Barry et al (2013) and Shearer et al (2021) examining the shape of the dose response based on untransformed serum PFOA as continuous exposure values, using the results of the 2-piece spline model for risk assessment, avoiding to choose a non-zero referent and analyzing specifically the low exposure range of the two study populations. The cohort data (n=32.254) of Barry et al. (2013) provided PFOA exposures and incident cancers among adults living near a chemical plant. Median concentrations measured 2005/2006 were 24.2 (0.25 – 4.752) ng/mL, estimated median annual levels were 19.4 (2.8 - 9.217) ng/mL for the community. The data were adapted by Steenland to parallel the NCI study design by Shearer et al (2021). A flattening / plateauing of the dose-response in the high exposure range was observed for the pooled data above the determined knot of the dose-response of about 10-13 ng/mL, meaning the dose-response is not linear, with a steep part in the low dose range (< 10-13 ng/mL). After converting to the absolute risk scale, the cancer slope factor of the steep low exposure range part of the 2-piece spline model was derived with 1.79x10^{-3} per ng/mL serum PFOA, applicable to the exposure range below the knot which is equal to the 85th percentile of the Shearer distribution and 60th percentile of the pooled data distribution. To the lower end, the PFOA exposure range extends approximately around 2 ng/mL: for Shearer et al., CalEPA (2024) has determined 2 ng/mL as the midpoint of the 25th percentile exposures of the study population. The PFOA concentrations estimated by Steenland et al. (2022) for the C8 population extends in the lower end as well towards 2 ng/mL.

PFOS cancer risk assessment

For PFOS the evidence for carcinogenicity is less strong. U.S. EPA and CalEPA did not identify epidemiological studies that are suitable for CSF derivation. CSF derivation based on animal data relies on the chronic rat study as described above and considered by IARC for classification (Butenhoff et al., (2012)).

Animal PoDs internal as calculated by EPA for rats are between 19.8 and 26.1 mg/L, reflecting much higher exposures than observed for PFOS in humans (e.g. comparing to Shearer et al. (2021), exposure quartiles for PFOS are: ≤26.3; > 26.3-38.4; >38.4-49.9; >49.9-154.2 µg/L).

4.4.3 Mode of Action considerations

While there is no doubt PFOA being carcinogenic in animals, the mechanistic evidence seems not sufficient and robust to describe the mode of action(s), its key events. In order to derive a threshold for carcinogenicity, the looming question is about PFOA genotoxicity. CalEPA considered there is evidence to suggest that PFOA may be genotoxic and has multiple key characteristics of carcinogens. U.S. EPA overall considers the evidence suggests that PFOA does not induce mutations or operate through a genotoxic mechanism, with the majority of the study data demonstrating a lack of genotoxic effect of PFOA in both *in vitro* and *in vivo* assays. Ultimately, based on the experimental and epidemiological evidence that was analyzed, no threshold was identified and both authorities apply a linear cancer risk extrapolation.

According to IARC, PFOA induces epigenetic alterations, induces oxidative stress, modulates receptor-mediated effects, furthermore PFOA alters cell proliferation, cell death, or nutrient supply and is immunosuppressive. IARC considers "there was suggestive evidence that PFOA is genotoxic. A single study in exposed humans reported increased levels of DNA strand breaks; results from other studies using less-relevant end-points were mixed. The results of studies in human primary cells were negative. Evidence in experimental systems suggested that PFOA causes DNA damage. Available studies in rodents *in vivo* showed largely negative results for DNA damage and micronucleus assays". Furthermore, there was suggestive evidence that PFOA induces chronic inflammation, and "there was a paucity of data for the following key characteristics: is electrophilic or metabolized to an electrophile, alters DNA repair or genomic instability, or causes immortalization".

4.4.4 Conclusions on available cancer risk assessments

PFOA carcinogenicity

It is noted that based on the key studies selected for the dose response analysis and derivation of cancer slope factors for PFOA as published by U.S. EPA (2024), CalEPA (2024) and Steenland (2022) for the observable exposure range in the general population, it may be concluded that the carcinogenicity is a (co)critical health effect of PFOA.

The IARC monograph considered studies published or accepted for publication in the openly available scientific literature by the time of the Working Group meeting, which was November 2023. Therefore, it is the most recent assessment based on a vast amount of data. Since then, few further studies have been published, raising concern for increased cancer risks to be associated with PFAS exposures; including (non-exhaustive) Biggeri et al (2024) reporting evidence of raised mortality from malignant neoplastic diseases, including kidney cancer and testicular cancer in PFAS-drinking water contaminated area in Italy and Sassano et al. (2025) publishing a systematic review and meta-analysis suggesting PFAS exposure may be associated with lung (occupational PFAS exposure) and thyroid cancer (PFOS).

The working group however acknowledges the inconsistency in the available data that deserve further attention and review. While the U.S. authorities based their cancer risk assessments on human data, in its monograph (IARC, 2025), IARC`s rational for classification of PFOA in the Group 1 is based on the combination of sufficient evidence for cancer in experimental animals and strong mechanistic evidence of key characteristics of carcinogens in exposed humans. The evidence for cancer in humans was found to be limited for renal cell carcinoma and cancer of the testis but the results were found to be inconsistent across the studies. For testicular cancer the positive findings were limited to two studies, one cohort study and a second ecological study with

limitations. For renal cell carcinoma, the positive findings observed in three in partly overlapping occupationally and environmentally exposed populations studies (Steenland and Woskie (2012); Vieira et al. (2013); Barry et al. (2013)) and in a fourth population with background exposure (Shearer et al. (2021)) study, contrast to two other background-exposed populations for which overall no such positive findings were observed (Rhee et al. (2023b), and Winquist et al. (2023). IARC therefore considered that "the body of epidemiological evidence indicated that a positive association between PFOA and renal cell carcinoma is credible, but positive findings have not been consistently observed among the most informative studies, and chance, bias, and confounding by other PFAS in some of the studies could not be ruled out with reasonable confidence. For other subtypes of kidney cancer, no conclusions could be drawn about an association with PFOA".

It is noted that it is exactly the two studies highlighted by IARC not reporting positive findings on kidney cancer that have been published more recently in 2023 ((Rhee et al. (2023b); Winquist et al. (2023)) and that were not considered in the U.S. EPA substance evaluation dossiers. It is further noted, that while animal data provide evidence for PFOA carcinogenicity, neither in rats nor in mice kidney tumors were found.

Therefore, the application of a cancer slope factor for the purpose of interpretation of HBM data in European population needs careful evaluation considering 1) the robust evidence in animals and Exposure-Risk-Relationships that maybe derived thereof; 2) human evidence with a credible positive association between PFOA and renal cell carcinoma, however, that is inconsistent regarding the overall epidemiological evidence, and slope factor derived as presented above; 3) the potential cancer mechanism of action(s) and the likelihood of a threshold below which cancer risk is unlikely or largely uncertain.

The working group felt that at current a proposal for a cancer slope factor is premature and requires in more in depths evaluation of the extensive IARC monograph and the epidemiological data presented, as well as the uncertainties associated with the application of cancer slope factor or a threshold.

As a first reflection on the cancer slope factors presented above, if using human data on RCC, the dose-response assessment approached by Steenland et al. (2022) would offer the shape of the linear spline in the observable low exposure range (≈ 2-10 ng/mL) and the so derived CSF of $1.79x10^{-3}$ ng/mL to be applied to the European HBM studies` exposure range limited to levels exceeding 2 ng/mL without uncertainties due to an extrapolation outside the estimated/measured exposures. For lower (< 2 ng/ml) PFOA serum concentrations extrapolation outside the "observable" range implicates that excess lifetime cancer risk are largely uncertain.

Increased OR of RCC were specifically observed in the Shearer et al. study for participants` ages 55–59 years, and in men and in women, separately. Such a CSF may be applied to the adult general population.

More recent estimated PFOA P50 serum concentrations in European adults (see HBM Dashboard) are below the lower exposure quartile values of both epidemiological studies considered by Steenland et al. (2022) for deriving the CSF based on pooled individual-level data (i.e. the P50 are < 4 ng/mL), specifically, the measured data in European populations is in the range of 1 to 3 ng/mL (see HBM4EU Dashboard), thus encompassing the 2 ng/mL midpoint of the Shearer`s NCI low exposure quartile.

For explorative purpose, and considering EU adult populations PFOA blood concentrations (HBM4EU Dashboard), the working group performed excess lifetime risk calculations for PFOA using a CSF of $1.79x10^{-3}$ ng/mL for serum levels that fall in the observable exposure range of the epidemiological studies considered by Steenland (2022), specifically in the linear low exposure part below the knot of the 2-piece spline model, i.e. ≈ within the 2 and 10 ng/mL:

PFOA levels were measured in European adults as published in the VITO HBM dashboard and provide percentiles for seven countries (DE, DK, CZ, BE, ES, AT, NO) for different sampling periods. Recent data for 2017 to 2019 show P50 of 0.8 to 1.9 ng/mL, and **P95 of 2.3 ng/mL** to 4.3 ng/mL (for 3 countries; DE, CZ, AT). Older available data for other countries (2010 to 2016) show P95 of 3.4 ng/mL and **6.3 ng/mL** and P50 of 1.6 and 2.9 ng/mL (DK and BE, respectively), data obtained before 2010 for two further countries show P95 close to 5 ng/mL (NO and ES).

Thus, European PFOA P95 exposure estimates are within the steep low exposure range part of the 2-piece spline model (2-10 ng/mL). European P95 serum concentrations would correspond to an estimated excess lifetime cancer risk in the range of $1x10^{-2}$ to $4x10^{-3}$ (P95 adults, 2010-2019):

$CSF_{internal}$ (Steenland et al., 2022): $1.79x10^{-3}$ $(ng/mL)^{-1}$

ExCR: 2.3 ng/mL x $1.79x10^{-3}$ $(ng/mL)^{-1}$ = $4x10^{-3}$

ExCR: 6.3 ng/mL x $1.79x10^{-3}$ $(ng/mL)^{-1}$ = $1x10^{-2}$

Such high end estimate of risk is an estimate only for those persons at the upper end of the risk distribution.

The HBM-I of 2 ng/mL would correspond to an excess lifetime cancer risk of $3.5x10^{-3}$:

$CSF_{internal}$ (Steenland et al., 2022): $1.79x10^{-3}$ $(ng/mL)^{-1}$

ExCR: 2 ng/mL x $1.79x10^{-3}$ $(ng/mL)^{-1}$ = $3.5x10^{-3}$

Following these calculations, statistically derived excess lifetime cancer risks for the specific subpopulation exceeding 2 ng/mL thus would be in the range of $3.5x10^{-3}$ to $1x10^{-2}$. More recent P50 estimates are below or close to 2 ng PFOA/mL serum and application of such CSF would be largely uncertain.

A quantitative cancer risk assessment would only be sought if a relevant percentile of the population exceeds the PFOA HBM-I value of 2 ng/mL.

PFOS carcinogenicity

The IARC Group 2B evaluation for PFOS is based on strong mechanistic evidence. IARC concluded there is strong evidence that PFOS exhibits multiple key characteristics of carcinogens in exposed humans, human primary cells, and experimental systems. In addition, the evidence for cancer in experimental animals was *limited*.

Considering this limited animal experimental data, the working group felt at present that the uncertainties are too high to consider PFOS carcinogenicity as an endpoint for HBM-GV derivation.

Interspecies extrapolation and the toxicokinetic differences between species implicate a major uncertainty, in addition if using animal data it does not reflect the human-relevant exposure conditions.

It is however acknowledged that tumor types observed, i.e. liver, maybe human-relevant tumor types/site for PFOS, as discussed by U.S. EPA considering the available epidemiological evidence.

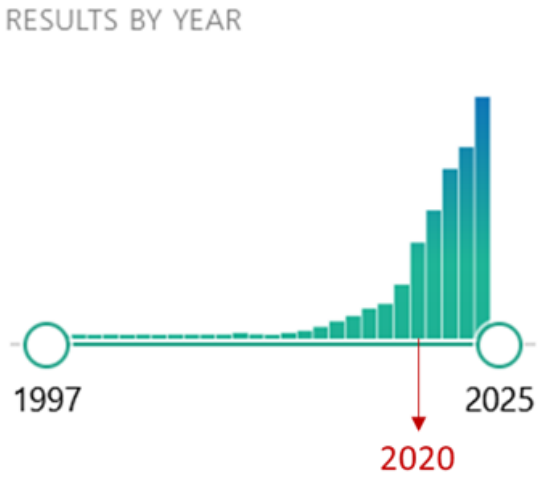
5 Literature review

The working group notes, based on an own literature search, that there is major epidemiological knowledge evolution in terms of published studies since EFSA (2020; literature search until August 2019). This concerns the non-carcinogenic critical health effects domains of PFAS, but also the carcinogenicity of PFOA with three of the human key studies considered by IARC published between 2020 and 2023. Given the large number of scientific articles published on PFAS and health effects since 2020 (over 1900 articles according to PubMed), the working group decided to focus first on reviewing systematic reviews and meta-analyses published to date. Meta-analyses and reviews allow to identify more accurate and reliable estimates of health effects by combining data from multiple studies, which improves statistical validity and reduces bias. As a result, this approach identified the health effects domains of PFAS that have been more extensively reviewed in the past years, as well the critical health effects domains for which there is higher evidence for causality

In Nov 2024, the working group identified 52 systematic reviews on PFAS health effects in PubMed. Especially since the year 2023 more systematic reviews and meta-analyses have been published, proportionate to the number of scientific articles published. After a first reading of the titles and abstracts, 9 systematic reviews/meta-analyses were excluded due to their lack of relevance to the topic/objective. From the 43 systematic reviews included, 25 were meta-analyses. Systematic reviews synthesize and summarize existing studies, while meta-analyses statistically combine data from multiple studies to provide quantitative estimate of effects. Ultimately, the working group focused on meta-analyses due to their ability to provide a more precise and reliable estimate of the effect size by pooling data from various studies. In Table XII.7, a summary of the identified meta-analyses is presented. The working group categorized the health outcomes into the following groups: cardio-metabolic, fertility and reproductive health, fetal growth and birth, immune system, thyroid function, liver damage, neurodevelopmental/neurological effects, and cancer. The working group summarized the evidence found in each meta-analyses and classified them as high, low, or moderate level of evidence. The evaluation of the level of evidence was based on the results of each association found in the meta-analyses. For example, if a meta-analysis found statistically significant negative associations across all exposure-outcome models, it was classified as having a 'high' level of evidence. However, the working group did not conduct a comprehensive analysis of the risk of bias within the meta-analyses.

Table XII.6: Count of scientific publications per year, PubMed (search term: "PFAS"; "health effects"; January 2025). Publications since year 2020 (year of EFSA evaluation).

Year	Count	Year	Count
2025	37	2013	9
2024	595	2012	4
2023	404	2010	2
2022	359	2009	4
2021	268	2008	1
2020	199	2007	1
2019	108	2006	1
2018	67	2005	1
2017	57	2003	1
2016	41	2001	1
2015	30	1999	1
2014	18	1997	1



In the table below, a summary of the meta-analyses published can be found.

Table XII.7: Overview of meta-analyses on PFAS, principal health effects in humans and level of evidence.

Author, year	Health outcome	Evidence	Level of evidence for an association
Gao X et al., 2021	Fetal growth and birth outcomes	A total of 29 studies (32,905 participants) were included. The pooled results demonstrated that **PFOS** exposure during pregnancy was associated with increased **preterm birth risk** (pooled OR per 1-ng/ml increase: 1.01, 95% CIs: 1.00-1.02, P = 0.009) and **PFNA** and **PFOA** exposure also showed associations with **preterm birth risk** (P values for the nonlinear trend: 0.025 and 0.030). Positive associations were also observed for exposure to **PFDA** and **miscarriage** (pooled OR per 1-ng/ml increase: 1.87, 95% CIs: 1.15-3.03) and **PFOS** and **preeclampsia** (pooled OR per 1-log increase: 1.27, 95% CIs: 1.06-1.51), whereas exposure to **PFUnDA** was inversely associated with **preeclampsia risk** (pooled OR per 1-log increase: 0.81, 95% CIs: 0.71-0.93). Based on individual evidence, detrimental effects were observed between **PFDA** exposure and **small for gestational age** and between **PFOA** and **PFOS** and **intrauterine growth restriction**. No significant associations were found between pregnancy PFAS exposure and other adverse pregnancy outcomes (i.e., gestational diabetes mellitus (GDM), pregnancy-induced hypertension, low birth weight, and large and small for gestational age).	Moderate
Si-Yu Gui et al., 2022	Fetal growth and birth outcomes	46 studies were included. Specifically, **birth weight** was associated with PFAS, with effect sizes ranging from -181.209 g (95% confidence interval (CI) = -360.620 to -1.798) per 1 ng/ml increase in **PFHpS** to -24.252 g (95% CI = -38.574 to -9.930) per 1 ln (ng/ml) increase in **PFDA**. Similar patterns were observed between other PFAS and birth outcomes: **PFOA** and **PFOS** with **birth length** and **ponderal index**, **PFOS** and **PFDoDA** with **head circumference**, **PFHpS** with **gestational age**, and **PFNA** and **PFHpS** with **preterm birth**. Additionally, **PFDA** showed a statistically significant association with **small for gestational age**.	High
J M Wright et al., 2023	Fetal growth and birth outcomes	A -32.9 g (95%CI: -47.0, -18.7) mean **birth weight** deficit per each ln **PFNA** increase was detected from 27 included studies. Contrary to earlier meta-analyses for PFOA and PFOS, birth weight deficits that were detected across all strata did not appear to be fully explained by potential bias due to pregnancy hemodynamics from sampling timing differences.	High
Lan L et al., 2023	Fetal growth and birth outcomes	A total of 21 studies were included. Among these studies, 18 studies examined the associations between **PFOA** and **birth weight**, 17 studies reported **PFOS**, and 11 studies discussed **PFHxS**. Associations between PFHxS (ES = -5.67, 95% CI: -33.92 to 22.59, P = 0.694) were weaker than those for PFOA and PFOS (ES = -58.62, 95% CI: -85.23 to -32.01, P < 0.001 for PFOA; ES = -54.75, 95% CI: -84.48 to -25.02, P < 0.001 for PFOS). The association was significantly stronger in the high median PFOS concentration group (ES = -107.23, 95% CI: -171.07 to -43.39, P < 0.001) than the lower one (ES = -29.15, 95% CI: -63.60 to -5.30, P = 0.097; meta-regression, P = 0.045). Limited evidence of a dose-response relationship between exposure to PFOS and infant birth weight was found.	Moderate
Hu C et al., 2023	Fetal growth and birth outcomes	Hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia. In total, 15 studies were included in the systematic review and meta-analysis. The results from meta-analyses showed that **risk of preeclampsia** was increased with exposure to **PFOA** (RR = 1.39, 95% CI = 1.05, 1.85; N = 6 studies; exposure = 1 ln-unit increment; low certainty), PFOS (RR = 1.51, 95% CI = 1.23, 1.86; N = 6 studies; exposure = 1 ln-unit increment; moderate certainty), and PFHxS (RR = 1.39, 95% CI = 1.10, 1.76; N = 6 studies; exposure = 1 ln-unit increment; low certainty). PFOS was also associated with an increased risk of hypertensive disorders of pregnancy (RR = 1.39, 95% CI = 1.10, 1.76; exposure = 1 ln-unit increment; low certainty).	Moderate

Author, year	Health outcome	Evidence	Level of evidence for an association
Stratakis N et al., 2022	Cardio-metabolic outcomes	Prenatal PFAS exposure with **BMI-z** in infancy showed a small inverse association with **PFOS** and **PFOA** (beta: −0.007; 95% CI: −0.012, −0.003; n = 3 estimates for PFOS and beta: 0.03; 95% CI: −0.02, 0.08; n = 3 estimates for PFOA). No significant associations between prenatal **PFOA** and **PFOS** with **childhood BMI** were found in meta-analyses. For adolescents, only two studies were available (no meta-analysis), they reported an increase in **obesity** when participants were more exposed to **PFOA** and **PFHxS**.	Low
Yan D et al., 2022	Cardio-metabolic outcomes	**PFASs** exposure was positively associated with the risk of **GDM**, with a summary OR of 1.09 (95% CI = 1.02-1.16; n = 11). Meanwhile, the summary OR from cohort studies for PFASs exposure was 1.06 (95% CI = 1.00-1.12; n = 8). The Beggs and Egger tests did not show publication bias, and the sensitivity analyses did not change the results in this meta-analysis.	High
Jeddi Z M et al., 2021	Cardio-metabolic outcomes	Five or more studies with exposure and outcomes assessments that were reasonably comparable. A total of 12 cross-sectional studies (10 studies on the general population and two studies in the occupational settings) investigated the association between PFAS exposure and metabolic syndrome. Pooled data from studies on the general population for **PFOA**, **PFOS**, **PFHxS** and **PFNA.** In the meta-analysis, the overall measure of effect was not statistically significant, showing no evidence of an association between concentrations of PFOA, PFOS, PFNA, and PFHxS and the **risk of metabolic syndrome.**	Low
Gui SY et al., 2023	Cardio-metabolic outcomes	22 studies were included. The meta-analyses indicated that the observed statistically significant PFAS-T2DM associations were consistent in cohort studies, while the associations were almost non-significant in case-control and cross-sectional studies. Dose-response meta-analysis showed a "parabolic-shaped" association between PFOA exposure and T2DM risk. Available evidence was rated with "low" risk of bias, and the level of evidence for **PFAS** and incident **T2DM** was considered "moderate".	Moderate
Pan K et al., 2023	Cardio-metabolic outcomes	13 studies were included. The results showed that **PFNA** (OR = 1.11, 95% CI: 1.04-1.19), **PFOA** (OR = 1.12, 95% CI: 1.02-1.23), **PFOS** (OR = 1.19, 95% CI: 1.06-1.34) and **PFHxS** (OR = 1.03, 95% CI: 1.00-1.06) were significantly associated with **hypertension**, while other types of PFASs (∑PFAS, PFDA, PFUnDA) had no statistical significance. In addition, PFNA (OR = 1.12, 95% CI: 1.03-1.22), PFOA (OR = 1.12, 95% CI: 1.01-1.25) and PFOS (OR = 1.12, 95% CI: 1.00-1.25) exposure were positively correlated with the risk of hypertension in men, but not in women.	Moderate
Frangione B et al., 2024	Cardio-metabolic outcomes	13 studies included in the meta-analysis. Prenatal exposures to **PFOS**, **PFOA**, **PFHxS** and **PFNA** were not statistically associated with changes in body mass index (**BMI**) or **waist circumference**. In contrast, postnatal exposures to **PFOS**, **PFOA**, and **PFHxS**, were inversely related to changes in **BMI** (i.e., per log10 increase in PFOS: BMI z-score of -0.16 (95% CI: -0.22, -0.10)). Our review found no evidence of a positive association between prenatal PFAS exposure and pediatric obesity, whereas an inverse association was found for postnatal exposure.	Moderate
Zheng Q et al., 2024	Cardio-metabolic outcomes	A total of 12 studies were included. There was a positive association between **PFAS** and **TC** (β = 1.110, 95% CI: 0.601, 1.610) and **LDL** (β = 1.900, 95% CI: 1.030, 2.770), and a negative association between **PFAS** and **HOMA-IR** in children and adolescents (β = -0.130, 95% CI: -0. 200, -0.059). **PFOS** was significant positive associated with **TC** (β = 8.22, 95% CI: 3.93, 12.51), **LDL** (β = (12.04, 95% CI: 5.08, 18.99), and **HOMA-IR** (β = -0.165, 95% CI: -0.292, -0.038).	High
Sun M et al., 2022	Thyroid function	A total of 32 observational studies were included. Prenatal exposure to **PFAS** (β coefficient, -0.017; 95% CI, -0.033 to 0) was negatively associated with neonatal **TT4** level. No associations for **TSH**, **TT3**, **FT3**, or **FT4**.	Moderate

Author, year	Health outcome	Evidence	Level of evidence for an association
Gerwen M et al., 2024	Thyroid function; Carcinogenesis	5 studies were included. Meta-analyses of **PFOA, PFOS, PFNA, PFHxS were not significant.**	Low
Du X et al., 2024	Thyroid function	Association between **16 PFAS compounds** and five thyroid hormones (**TSH**, **TT3**, **TT4**, **FT3**, and **FT4**) in the serum of a pregnant women, adolescents, and adults. The dose-response relationship between some PFAS and thyroid hormones in different population subpopulation was found and the model was fitted. Data from 18 animal experiments with previously published in vitro studies was used to elucidate the toxicological mechanisms underlying the impact of PFAS on the thyroid gland. The results of the study showed that (a) both conventional and emerging PFAS compounds were identified in human samples and exhibited associations with thyroid health outcomes; (b) in animal studies, PFAS have been found to impact thyroid gland health through two primary mechanisms: by influencing the hypothalamic-pituitary-thyroid axis and by binding to thyroid receptors.	Moderate
Costello E et al., 2022	Liver damage	85 rodent studies and 24 epidemiological studies were identified. Studies focused primarily on legacy PFAS: **PFOA**, **PFOS**, **PFNA,** and **PFHxS.** Meta-analyses of human studies revealed that **higher ALT levels** were associated with exposure to **PFOA** (z-score= 6.20, p<0.001), **PFOS** (z-score= 3.55, p<0.001), and **PFNA** (z-score= 2.27, p=0.023). **PFOA** exposure was also associated with **higher aspartate aminotransferase** and **gamma-glutamyl transferase** levels in humans. In rodents, PFAS exposures consistently resulted in higher ALT levels and steatosis.	High
Zhang X et al., 2022	Immune system	9 studies about children met inclusion criteria. A high degree of heterogeneity is observed in terms of breastfeeding time, confounder control, and detection method. Exposure to **PFOA** and **PFHxS** is negatively associated with **tetanus antibody level** in children without heterogeneity by Cochran's Q test (p = 0.26; p = 0.55), and exposure to **PFHxS** is negatively associated with **diphtheria antibody level** but with heterogeneity (p = 0.04). For **PFOS**, **PFDA**, and **PFNA**, associations were not found. The association of the **other** four **vaccines** (measles, rubella, mumps, and influenza) with PFAS remains uncertain because **very few studies are available.**	Moderate
Crawford L et al., 2023	Immune system	The 14 studies represented 13 unique groups of subjects; the frequency of studies of a given **antibody** was Tetanus (n = 7); followed by Diphtheria (6); Measles (4); Rubella (3); Haemophilus influenzae type b and Influenza A H1N1 (2 each); and Hepatitis A, Hepatitis B, Influenza A H2N3, Influenza B, and Mumps (1 each). The summary associations (coefficients for difference in loge[antibody concentration] per doubling of serum PFAS) with 95% confidence intervals that excluded zero ("statistical support"), and certainty of evidence ratings were as follows: for **PFOA** and all antibodies treated as **one type** in all ages, -0.06 (-0.10, -0.01; moderate) and in children, -0.10 (-0.16, -0.03; moderate); for **Diphtheria** in children, -0.12 (-0.23, -0.00; high); for **Rubella** in all ages, -0.09 (-0.17, -0.01; moderate), and for **Tetanus** in children, -0.12 (-0.24, -0.00; moderate). For PFOS the summary associations were, for all antibodies treated as **one type** in all ages, -0.06 (-0.11, -0.01; moderate) and in children, -0.10 (-0.18, -0.03; moderate); for **Rubella** in all ages, -0.09 (-0.15, -0.03; high) and in children, -0.12 (-0.20, -0.04; high). For **PFHxS** the summary associations were, for all antibodies treated as **one type** in all ages, -0.03 (-0.06, -0.00; moderate) and in children, -0.05 (-0.09, -0.00; low); and for **Rubella** in children, -0.07 (-0.11, -0.02; high). Summary associations for **PFNA** and **PFDA** did not have statistical support, but **tended** to have an **inverse association with antibody concentrations.**	High

Author, year	Health outcome	Evidence	Level of evidence for an association
Antoniou E et al., 2024	Immune system	The aim of this study was to examine the effects of PFAS exposure on vaccine antibody response and risk of infection in humans. 17 studies were included. This meta-analysis summarizes the findings of PFAS effects on infant and childhood immune health. The **immunosuppression findings for infections** yielded suggestive evidence related to PFAS exposure, particularly **PFOS**, **PFOA**, **PFHxS,** and **PFNA** but moderate to no evidence regarding antibody titer reduction.	Moderate
Wang W et al., 2023	Fertility and reproductive outcomes	13 articles were included. Meta-analysis showed that **PFOA** exposure was negatively associated with the **female fecundability** odds ratio (FOR = 0.88, 95% confidence interval (Cl) [0.78; 0.98]) and positively associated with the odds ratio for infertility (OR = 1.33, 95%Cl [1.03; 1.73]). **PFOS** exposure was negatively associated with the **fecundability** odds ratio (FOR = 0.94, 95% CI [0.90; 0.98]). Pooled effect values for **PFNA**, **PFDA**, and **PFHxS** exposure did **not find sufficient evidence for an association with female fertility.**	Moderate
Li L et al., 2024	Fertility and reproductive outcomes	11 studies involving 7714 participants were included. Meta-analysis showed that **PFHxS** exposure was positively associated with **estradiol levels** in **female** [β = 0.030, 95% CI: (0.013, 0.046), P = 0.000]. A negative association was found between **PFOA** [β = -0.012, 95% CI: (-0.023, -0.002), P = 0.017] and **PFOS** [β = -0.011; 95% CI: (-0.021, -0.000), P = 0.042] exposure with **male testosterone** levels. In the subgroup analysis, there were stronger associations in children than in adults. And the high heterogeneity was mainly due to the cross-sectional studies. Publication bias was not found.	High
Cong X et al., 2023	Carcinogenesis (breast cancer)	A total of 17 publications were included for quantitative evaluation. Meta-analysis showed that **PFOA** (OR = 1.07, 95% CI = 0.84-1.38, I2 = 85.9%, P < 0.001), **PFOS** (OR = 1.01, 95% CI = 0.95-1.08, I2 = 65.7%, P < 0.001), **PFNA** (OR = 0.89, 95% CI = 0.67-1.19, I2 = 74.4%, P < 0.001), and **PFHxS** (OR = 0.90, 95% CI = 0.72-1.13, I2 = 74%, P < 0.001) were **not significantly correlated with breast cancer.**	Low
Liu D et al., 2024	Neurodevelopmental/Neurological	A total of 29 publications were included in the meta-analyses. PFAS and child Intelligence Quotient (IQ), executive function and behavioral difficulty. There is evidence of sex-specific relationship between PFAS exposure and children's PIQ. We found that **PFOS** [β = -1.56, 95% CI = -2.96, - 0.07; exposure = per 1 ln (ng/ml) increase], **PFOA** [β = -1.87, 95% CI = -3.29, - 0.46; exposure = per 1 ln (ng/ml) increase], **PFHxS** [β = -2.02, 95% CI = -3.23, - 0.81; exposure = per 1 ln (ng/ml) increase] **decreased performance IQ** in **boys**, but **PFOS** [β = 1.56, 95% CI = 0.06, 3.06; exposure = per 1 ln (ng/ml) increase] **increased performance IQ** in **girls**. PFAS are associated with executive function impairments in children, but not related to behavioral difficulty in children.	Moderate
Khosrojerdi M A et al., 2024	Osteoporosis	10 studies were included in the final meta-analysis. Serum **PFOA** (β = -0.11, 95% confidence interval (CI): -0.18, -0.05) and **PFOS** (β = -0.06, 95% CI: -0.11, -0.01) levels showed significant negative **correlations with total body less head bone mineral density.** Subgrouping showed that only **PFHxS** (odds ratio [OR] = 1.37, 95% CI: 1.12, 1.68) was correlated with **osteoporosis**.	High
Chen J et al., 2023	Oxidative stress	A total of 49 studies were selected for the PFAS meta-analyses. We found consistent positive associations of **PFAS** with **GGT** and **uric acid**. For the other markers, PFAS were not associated.	Moderate

The working group identified that the health outcomes with more meta-analyses published were cardio-metabolic outcomes, fetal growth and birth outcomes, immune system, and thyroid function, with 7, 5, 3, and 3 meta-analyses, respectively. According to the conclusions of each analysis, the health outcomes with highest levels of evidence for associations were the following: immune system, fertility and reproductive outcomes, cardio-metabolic outcomes, and fetal growth and birth outcomes.

Therefore, the working group considers it necessary to pay further attention to the question whether the EFSA-4 is protective for all effect domains based on most recent knowledge.

The U.S. EPA (2024) considers the endpoints immunological effects, development and cholesterol as co-critical. The EPA assessment is based on literature search conducted finally until February 2023, the IARC covers literature until November 2023.

It is also noted that the critical health effects domain “developmental effects” (↓Birth weight) is relevant for HBM-GV derivation of women in reproductive age, while cardiovascular effects (↑Cholesterol) are relevant for HBM-GV for the general population considering all subpopulations (similarly, PFOA carcinogenicity risk for RCC would be relevant for the adult population). The EFSA derived a sum value based on the health effect domain “immunological effects” (↓serum Ig) is meant to be protective for all population groups. In principle distinct values may be derived or may need to be derived depending on the endpoint or the subpopulation at potential risk.

It is furthermore noted that for other individual PFAS substances knowledge evolution may at some point allow the derivation of values with sufficient confidence.

6 Recommendation for further work

The following table consolidates an overview of the available assessments and reviews presented above for all substances in the Aligned studies scope for non-cancer endpoints and carcinogenicity. Based on the review and consolidation, the working group has the following recommendations for the second phase of the project P.4.1.3.1.a_HBM-GV as regards to PFAS HBM-GV derivation.

Further work is suggested especially for substances that have a relevant contribution to PFAS exposure and where new epidemiological data became available in recent years (after 2020).

The working group concluded that derivation of HBM-GV for the full scope of PFAS compounds measured in the PARC aligned studies may not be justified due to their minor or negligible contribution to exposure.

Substances that have only minor or uncertain/unknown relative contribution or low detection frequencies, may have less priority in future work, in particular where epidemiological data are scarce or animal data are limited and implicate high uncertainties in a risk assessment.

Regarding the use of animal data, the working groups considers the added value of deriving substance specific HBM-GV for PFAS compounds limited, as the overall precision of the PFAS risk assessment likely will not significantly increase, considering high assessment factors suggested on the on hand (see UBA, 2023: total AF of 400 to 144 200), and their minor or even negligible contribution to PFAS body burden on the other hand. Notably, for none of the PFAS to be analysed in PARC Aligned studies, UBA identified a long-term toxicity study and only very few of the identified studies suggest adverse effects of the critical human effect clusters as underlying the PoD. However, eight substances of the PARC Aligned studies scope were not included in the review by UBA

Based on our preliminary review of epidemiological literature presented in this scoping document (meta-analyses and reviews, studies identified by UBA, 2023), we suggest to continue with a targeted review of epidemiological literature for the critical effect domains identified above, namely immune system, fertility and reproductive outcomes, cardio-metabolic outcomes, and fetal growth and birth outcomes. This target literature review may encompass the studies identified by UBA (2023) and which have been evaluated by us as medium or high confidence, furthermore recent studies of similar confidence and reviewed by IARC (2025), as well literature for the most recent time period that has not been covered by any of the previous assessments (EPA, IARC, UBA).

The working group specifically suggests to review the evidence for causality for increased cholesterol associated with PFOS and PFOA exposure. Diverging opinions have been published in this matter by EFSA (2020), contrasted by U.S. EPA, CalEPA (2024), and IARC (2025).

This literature review may focus on those substances included in the PARC Aligned studies for which relevant exposures have been reported in Human Biomonitoring studies, thus may primarily focus on the EFSA-4 (PFOA, PFOS, PFNA, PFHXs) with a view to analyses whether the EFSA Sum values are still appropriate.

After identifying potential key studies, BMD modelling may be needed to derive a PoD.

Specifically on PFOA, the working group suggests to review the carcinogenicity database in more detail, considering the recently published IARC Monograph and latest literature, in order to conclude whether it is appropriate to recommend a cancer slope factor. This should encompass mechanistic evidence and the question of a threshold or non-threshold mechanism (genotoxicity).

Considering its high contribution to PFAS body burden, a review of recent toxicological and epidemiological evidence for carcinogenicity of PFOS, and whether further data would support to derive a CSF for cancer risk assessment, may be envisaged.

As mentioned in the introduction chapter, PARC includes also targeted studies on PFAS in children and studies in pregnant women, breastmilk and newborns. These studies start in the second phase of PARC (Y4; May 2025). The substance scope is not defined yet. Sampling matrices suggested include breastmilk, urine, blood and others. Furthermore, PFAS are also included in PARC occupational biomonitoring studies. In the second phase of P.4.1.3.1.a_HBM-GV, further PFAS, including short-chain PFAS may be prioritized beyond the PARC aligned study scope of the General population survey.

Table XII.8: Overview of available assessments and reviews for PFAS substances included in the Aligned studies scope and recommendations for future work under P.4.1.3.1.a_HBM-GV

	Available assesments	non-cancer									carcinogenicity			HBM4EU teenagers		Recommendation	Justification
		EFSA (2020)	German HBM Commission (2016-2021)	UBA (proposal HBM-I/II) (2020)	UBA literature review (proposal DWLW) (2023)	HBM4EU (proposal HBM-GV) (2020)	US EPA (2021/2024)	CalEPA (2024)	Health Canada (2018)	ATSDR (2021)	US EPA (2024)	CalEPA (2024)	Steenland (2022)	Average percentage Detection Frequency (blood)	Median concentration contribution (blood)		
PARC GS aligned scope	PFOA	human	human		new epi		human	human	animal****	animal*	human	human	human	98,4%	26,8%	review : cancer MoA/genotoxicity; epidemiological evidence, causality cholesterol	New data available, the substance has a relevant contribution
	PFOS	human	human		new epi		human	human	animal****	animal*	animal	animal		100%	54,4%	review : carcinogenicity; epidemiological evidence, causality cholesterol	New data available, the substance has a relevant contribution
	PFNA	human			new epi					animal*				85,6%	7,0%	review : carcinogenicity; epidemiological evidence	New data available, the substance has a relevant contribution
	PFHXs	human			new epi	animal				animal*				86,4%	8,8%	review : epidemiological evidence	New data available, the substance has a relevant contribution
	PFDA				animal									62,4%	1,5%	no action	High animal-human extrapolation uncertainties and minor contribution
	PFBS			animal***	animal		animal**			data gaps				4,0%	<LOQ	no action	High animal-human extrapolation uncertainties and uncertain contribution
	PFHpS				data gaps									45,6%	0,2%	no action	Data gaps, minor contribution
PARC GS discovery scope	4:2 FTSA													uncertain	uncertain	no action	Uncertain contribution
	6:2 FTSA													uncertain	uncertain	no action	Uncertain contribution
	8:2 FTSA													uncertain	uncertain	no action	Uncertain contribution
	9CL-PF3ONS													uncertain	uncertain	no action	Uncertain contribution
	PFHxA			animal***	animal					data gaps				27,3%	0,3%	no action	High animal-human extrapolation uncertainties and minor contribution
	PFHpA				animal					data gaps				26,7%	0,3%	no action	High animal-human extrapolation uncertainties and minor contribution
	PFDoDA				animal					data gaps				4,2%	<LOQ	no action	High animal-human extrapolation uncertainties and uncertain contribution
	PFUnDA				animal									50,7%	0,5%	no action	High animal-human extrapolation uncertainties and minor contribution
	PFPeA				data gaps									19,9%	0,2%	no action	Data gaps, minor contribution
	PFBA			animal***	new epi					data gaps				uncertain	uncertain	tbd review : epidemiological evidence	Uncertain contribution, but epidemiological data identified
	PFNS				data gaps									uncertain	uncertain	no action	Uncertain contribution
	EtFOSAA													uncertain	uncertain	no action	Uncertain contribution
	MeFOSAA													uncertain	uncertain	no action	Uncertain contribution
	Adona													uncertain	uncertain	no action	Uncertain contribution
	GenX						animal							uncertain	uncertain	no action	Uncertain contribution

non-cancer endpoints

PoD animal data

AF proposed:

<100

100-500

500-1000

>1000

>10000

> 100000

PoD human data

human data

carcinogenicity

CSF based on animal data

animal

CSF based on human data

human

* ATSDR derived intermdiate-duration MRL, no chronic MRL due to lack of appropriate studies

** "low confidence" assigned by EPA

*** labelled "provisionally" due to uncertainties in interspecies extrapolation

**** considered outdated

data gaps

new epi data

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Part XIII

Status paper - **Arsenic** and its inorganic compounds

Status paper – Arsenic and its inorganic compounds

The derivation of Human Biomonitoring Guidance Values (HBM-GVs) for arsenic and its inorganic compounds, for the general population and the workers is currently underway by ANSES (FR).

This work involves a review of the literature and a rigorous scientific evaluation of the existing data in view of establishing a toxicological profile. On this basis, the derivation of the guidance value will be made after selecting the biomarker of exposure, the critical effect and the key study /studies.

This arsenic report was originally due to be included in this Additional Deliverable (AD) at Month 36 of PARC, but delays have been encountered. This section of the AD provides a brief overview of the work to date, an update on progress and next steps which will be completed and published in the next AD.

Current Status of Work

Initially, a literature search using the PubMed and Scopus databases was carried out in July 2024 to supplement the data in the ATSDR 2016 and EFSA 2024 reports. Indeed, occupational exposure studies were not included in the EFSA report. This initial search resulted in 6,510 publications, which were initially screened using ASReview (AI-aided Open-Source Systematic Review Software). This literature update is currently being finalised, and each publication is being assessed by two independent reviewers.

Furthermore, in January 2025, the U.S. EPA published its final report on inorganic arsenic, which will also be taken into account when establishing the toxicological profile. This report includes the derivation of reference doses (RfDs) for specific endpoints following chronic oral exposure to arsenic as well as cancer slope factors (CSFs) for non-threshold carcinogenic effects (U.S. EPA. 2025).

It is important to note that, for inorganic arsenic, a large amount of data and publications need to be taken into account and evaluated for the derivation of the HBM-GVs. This wealth of information, while valuable, also contributes to the complexity and additional time required.

At this stage, the first introductory parts of the report have been finalised and include: physico-chemical characteristics of the different inorganic compounds; use and sources of exposure; kinetics and physiologically based kinetics (PBK) modelling; identification of possible biomarkers of exposure; urinary levels reported in biomonitoring campaigns; reporting of existing toxicological reference values for the general population and workers.

We still need to finalise the literature update and data analysis in order to complete the toxicological profile, choose the derivation approach for the general population and workers, and derive the scientifically-based HBM-GVs, in order to have a first draft available for consultation.

Next Steps

Once finalised, the draft will be shared between the project partners, after which the National Hubs will be consulted. Feedback and suggestions will be taken into account where appropriate and documented transparently. The final report detailing the HBM-GV derivation will therefore be included in the next AD scheduled for month 60 of PARC, i.e., April 2027.

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Part XIV (ANNEX)

List of comments for Parts I to XIb

1 Annex: List of comments and how they were taken into account

1.1 Part I - DHHB

Part I - DHHB			
Version	Date of receipt of opinion	Reviewers (only the hub affiliation specified)	Comments and short description of changes
1	08.03.2023	Project partner Nancy Hopf/Unisanté	Inclusion of potential occupational exposure (now in 12: Annex on occupational exposure)
2	30.06.2023	Swedish NH	Inclusion of explanation why no HBM-GV for workers has been derived so far. In the extension period of project P4.1.3.1.a (year 4 or 5) it will be examined whether it is possible to derive an HBM-GV for workers for this substance, in this case the derivation will be carried out.
2	11.07.2023	German NH	Abstract: Modification of abstract (now paragraph on UVINUL A Plus included); II Background Inclusion of explanation why no HBM-GV_{worker} has been derived so far. In the extension period of project P4.1.3.1.a (year 4 or 5) it will be examined whether it is possible to derive an HBM-GV for workers for this substance, in this case the derivation will be carried out ; 1 Introduction: Inclusion of Stoeckelhuber et al. (2020) in section on information used for the assessment; 5 Exposure: Inclusion of exposure data from Stoeckelhuber et al. (2019) in exposure section; 6 Toxicological profile: In the toxicokinetic section the exact measured values are now given (instead of rounded values); The sentence giving the factor of dermal absorption in vitro in comparison to the in vivo data is deleted as suggested; 8 Selected Biomarkers: It is now explained in more detail why the HBM-GVs were derived only for AHB and EHB (and not for DHB); Urinary concentrations are now given in µg/L (instead of µg/g creatinine).

Part I - DHHB			
Version	Date of receipt of opinion	Reviewers (only the hub affiliation specified)	Comments and short description of changes
2	27 July 2023	UK NH	The minor comments have mostly already been considered in the draft. It is now explained why effects observed in animal studies can also be assumed for humans. The rationale for choosing DHHB for the derivation of HBM-GVs has already been included in the introduction (i.e. high production volume and high frequency of detection). Spelling mistake "EHP" now corrected to "EHB" A reference for different exposure pathways in humans is now given. The allocation of AFs is based on ECHA R.8 and expert judgement.
2		Austrian NH	Abstract: focus on DHHB, now redrafted; II Background: time frame of ESB sampling, reference for kinetic studies, chemical names of metabolites: all changed/included now accordingly; Explanation given why no HBM-GV was derived for workers; Glossary/Abbreviations: text for AFs now in footnote at the corresponding page 1 Introduction: Information on how long DHHB is used in the applications listed, and introduction on market now given; Description of the approach of the literature search now included; 2 General information and physical-chemical properties of DHHB: Suggested modifications accepted; Paragraph on degradation: modified; section supplemented with partition coefficient octanol/air and octanol/water; 3 Occurrence and use of DHHB Directive now quoted with number; The occurrence of DHHB in the cosmetic products was self-declared (now included); Information on DHHB content of products could not be included, since this is not presented in Danish EPA, 2015; It is now highlighted that DHHB is often used with other UV filters in combination and references are provided; 4 EU legislation and regulation, classification; Changed to: legal framework; 5 Exposure of the general population:

Part I - DHHB			
Version	Date of receipt of opinion	Reviewers (only the hub affiliation specified)	Comments and short description of changes
			Now added that also from manufacturing and subsequent downstream activities environmental exposure is possible/relevant; Sentence rewritten, so that it is now clear that the use of cosmetic products is the most important source for environmental exposure; 6.2.2 Irritation and sensitization The two studies are brought into context and the % positive results is now reported; 6.2.3.2 Animal data Any other investigation concerning immunotoxicity e.g. white blood cells number or phenotyping performed? There are no treatment-related changes in the hematology parameters measured. Increased liver weight changes ≤10% can be an adaptive response and are not necessarily adverse. Yes, therefore a NOEL was derived and not a NOAEL Without a histopathological correlate? Yes, now information included in the text. 6.2.7 Mechanistic findings It was noted that the uterotrophic assay is only validated for agonistic E effects. The text was changed as suggested. "Due to a limited database, further investigations could not conclude whether DHHB is an endocrine disruptor or not (Axelstad et al.; Danish EPA, 2015; Maipas and Nicolopoulou-Stamati, 2015)" This statement is not fully understandable having an OECD level 5 study (OECD TG 416 protocol 2001) and two negative mechanistic in vivo assays available. Agreement, references do not provide sufficient justification, therefore note deleted 9.1 Choice of key study, POD and assessment factors Developmental effects are named and the choice of the key study and effect is explained more clearly: The key study is reliable without restrictions and detailed investigations have been performed; furthermore, it is a two-generation study. Table 10: Data sheet for proposed derivation of HBM-GVs for DHHB Suggested modification accepted, "several" was changed to "two"; As there is no reliable dermal toxicity study available nor a conversion factor from oral to dermal, it had to be assumed that absorption and metabolism are the same in both routes.

Part I - DHHB			
Version	Date of receipt of opinion	Reviewers (only the hub affiliation specified)	Comments and short description of changes
3	January 2025	Adoption by P4.1.3.1.a partners, comment by partner Tamar Berman (MOH-IL) has been taken into account	Clarification adverse effects
4 Final Version	January 2025		

1.2 Part II - (λ- and γ-) cyhalothrin

Part II - (λ- and γ-) cyhalothrin			
Version	Date receipt of opinion	Reviewer (only the hub affiliation or European Agency specified)	Comments and short description of changes
Version 2	30.11.2023	German NH	Overall, a very good and coherent derivation, also very well written. Thank you. Basically, however, I wanted to point out again to prevent future misunderstandings, that the total Fue must generally be used for the derivation (steady state assumption), and not (!) the 24-h Fue. In most cases of the current substances (as well as here) this makes little difference, as 90-95% is excreted within the first 24 hours. The somewhat smaller 24-h Fue leads to 5-10% more 'conservative' results in the back-extrapolation, which is pi-mal-thumb protective and therefore OK. For other substances with somewhat delayed excretion (e.g. alkylpyrrolidones), however, it would lead to a considerable distortion, which is not correct. This point has now been taken into account.
Version 2	30.11.2023	German NH	Information on regulatory aspects und correction of biomonitoring data. Taken into consideration, thank you.
Version 2	15.12.2023	Swiss NH	Overall, I found the derivation comprehensible and appreciated the confidence assessment of critical studies. Thank you. Why not also an HBM guidance value for worker was derived? Will be added in the second period of the project.
Version 2	20.12.2023	Slovakian NH	Page 16: add reference to U.S. EPA Office of Pesticide Programs A Review of the Relationship between Pyrethrins, Pyrethroid Exposure and Asthma and Allergies, September 2009, corrected version. Thank you for the hint, done
Version 2	20.12.2023	German NH	It should be explained in greater detail how HBM-GVs are related to Health-based guidance values (HBGVs). Done Is it appropriate (and why) to estimate oral absorption in man on the basis of CIF3CA excretion in urine. Is it possible that this approach might result in underestimation when the other metabolites are left aside? CIF3CA is a largely specific metabolite of cyhalothrin (see explanations in the text), the excretion of which was determined in relation to the amount of cyhalothrin administered orally. For this reason, an underestimation of the oral exposure cannot be assumed. Acute toxicity – Animal studies: Give data obtained with racemic cyhalothrin. Done Regarding gamma-cyhalothrin, it should be mentioned if the "transient clinical signs" were related to administration via all routes or confined to the dermal study that is briefly reported immediately before. Statements on temporary clinical signs were removed, as they are not relevant here.

Part II - (λ- and γ-) cyhalothrin			
Version	Date receipt of opinion	Reviewer (only the hub affiliation or European Agency specified)	Comments and short description of changes
			Long-term studies and Carcinogenicity – Animals studies: Apparently, the same long-term study for chronic toxicity and carcinogenicity in rats is reported twice, put considerations on study reliability in the chronic toxicity part. Info added also there. With regard to carcinogenicity, reliability mainly depends on the availability of all animals (including premature decedents) for comprehensive histopathological evaluation. If this was the case, the conclusion that there was no evidence of carcinogenicity appears robust. The assessment of official bodies has been adopted here. For the long-term study in mice, an incidence of 1.9% for mammary gland adenocarcinoma in females is reported for the concurrent control. In the mid and high dose groups, the incidences were 13.5% and 11.5%, respectively, and, accordingly, in the magnitude of the upper edge of the historical control incidence. The latter may be true but certainly the incidences at the mid and high dose levels were not "similar to the experimental control" as claimed in the respective paragraph on page 23 but much higher. Yes, after the quote it should read "historical control": "the concurrent control incidence is low (1.9 %) and lies outside the historical control range….." Toxicity to reproduction – Animal studies: What does "FAO/WHO (2007)" in the first paragraph mean? Was it JMPR, too? FAO/WHO (2009) in the subsequent paragraph is certainly "JMPR, 2009", isn't it? Thank you for the hint. I checked the citation and corrected to JMPR 2007. Where does the NOAEL of 0.5 mg/kg bw per day come from that was apparently used as basis for deriving the ADI? The doses given are somewhat different in the various reports and cannot be clearly clarified despite intensive research. Only EFSA can be quoted here. Role of an RMS? Given in the list of abbreviations What are the "internal concentrations" in the neurotoxicity study by Moser et al. (2016) and how are they determined? Concentrations given, method mentioned What neuropathological lesions were observed in the DNT study at the high dose? Added Derivation of a human biomonitoring guidance value for CIF3CA: even though the approach taken appears reasonable, some questions still remain open and should be discussed before it can be eventually adopted. The same method was used as by Tarazona et al. (2022) reporting findings from the HBM4EU initiative but the outcome of the calculation is different because of a different urine volume, when adjusted for bodyweight, for children (i.e., 0.03 L/kg bw) and for adults (i.e., 0.02 L/kg bw). All HMB-GVs that were developed within HBM4EU, either adopted (cyfluthrin, deltamethrin) or proposed, were derived under this assumption. If a new assumption of a "standard" urinary volume of 0.03 L/kg bw for all is accepted, all the adult-specific HBM-GVs must be deleted and there will be left only one numeric value for every metabolite and/or active substance that is, for adults, lower than before. This should be officially agreed on EU level. For doing so, we must be sure about science. We have now decided to use the old values until the updated urine volumes are published and then to update all values derived up to that point if necessary. For

Part II - (λ- and γ-) cyhalothrin			
Version	Date receipt of opinion	Reviewer (only the hub affiliation or European Agency specified)	Comments and short description of changes
			information purposes, we will provide a reference to the ongoing work and point out that the values derived now may change. The other main difference is specific for cyhalothrin and consists in the use of an ADI of 0.0012 mg/kg bw for gamma-cyhalothrin in the equation. Its experimental basis is the same as for the ADI of 0.0025 mg/kg bw for lambda-cyhalothrin, i.e., the NOAEL of 0.5 mg/kg bw per day in the same multigeneration study with cyhalothrin in rats. However, the uncertainty factors for the two isomers were different (400 vs. 200). In the article by Tarazona et al. (2022), all considerations were confined to lambda-cyhalothrin and the gamma isomer was even not mentioned. It seems that only lambda-cyhalothrin was on the list of "pyrethroids of interest" within HBM4EU. Since gamma-cyhalothrin is still an approved pesticide in the EU, the approach taken by Apel and Voss (2023) in this document is supported. Ok Further editorial remarks were taken into consideration.
Version 2	22.12.2023	Swiss NH	A more comprehensive graphic than Figure 1 would be nice. At this point, the graphic is only intended to provide a general overview. New data is available on OPEN EFSA for lambda cyhalothrin (for example, in vitro comparative metabolism study in microsomes). Although this data has not yet been peer reviewed, would it be possible to include the data or include it once it has been peer reviewed? Once it has been peer reviewed it should be included. Additional information which (OECD) Guideline was followed would be nice to have in the tables. Added As the original data is not available to the authors, would this not impact the level of confidence? Also, the ADIs for lambda- and gamma-Cyhalothrin was determined using a study performed which cyhalothrin, and then adding additional uncertainty factors. Would this not also impact the level of confidence? Yes, level of confidence downgraded to "medium". A weighing of the various factors influencing the final level of confidence would be nice. Information given.
Version 2	05.01.2024	UK NH	Why was urine volume in ml used and not urine creatinine concentration? According to Abraham et al. (2023) comparing vegans, vegetarians and omnivores, there are risks of severe misinterpretations if biomarker measurements in spot urine are adjusted for creatinine. I have included a corresponding justification in the text. "Justification for the change in the basis of calculation will be published separately." (footnote 6, p29) – this is not referenced, which to my mind should be rectified. We have now decided to use the old values until the updated urine

Part II - (λ- and γ-) cyhalothrin			
Version	Date receipt of opinion	Reviewer (only the hub affiliation or European Agency specified)	Comments and short description of changes
			volumes are published and then to update all values derived up to that point if necessary. For information purposes, we will provide a reference to the ongoing work and point out that the values derived now may change. Estimated NOAEL of 0.5 mg cyhalothrin/kg bw/day used in the ADI derivation (which wasn't explained by EFSA): is it possible we could probe further on how EFSA derived this value? The doses given are somewhat different in the various reports and cannot be clearly clarified despite intensive research. Only EFSA can be quoted here.
Version 2	05.01.2024	Spanish NH	It should be noted that it is stated that gamma cyhalothrin is the most toxicologically active component, however the data from the repeated dose toxicity studies report lower NOAEL values for lambda cyhalothrin. Therefore, the higher toxicity of gamma-cyhalothrin could only be asserted in the context of acute toxicity. Thank you for the hint, corrected In addition, long-term studies should be considered with caution, they have been conducted with cyhalothrin (but not with lambda- or gamma-cyhalothrin) and higher NOAELs are obtained than those reported for gamma- or lamda-cyhalothrin in short-term studies. Considered Reference(s) justifying the use of the new default urinary volume value for adults should be included. So far, there is only a publication draft of the German HBM Commission, so that we have decided - also for reasons of consistency with regard to the values already derived under HBM4EU - to work with the previous values first until publication, but to already give an indication of possible changes.
		Spanish NH	Please add in table 3, line 1 to clinical signs a reduced weight gain and food consumption and alterations of clinical-chemical parameters. Done Further editorial remarks were taken into consideration.
		Spanish NH	"It is concluded that ClF3CA in urine reflects prior exposure to cyhalothrin, at least in Germany and probably also in other countries of the European Union." This statement seems to lack support with evidence, there's only this paper by Schettgen analyzing both metabolites. Second half-sentence deleted and added: "Moreover, bifenthrin is currently not approved as insecticide in the European Union." Further editorial remarks were taken into consideration.
Version 2	17.01.2024	Austrian NH	Suggested corrections to regulation and monitoring topics have been adopted. Thank you very much for the information. Further editorial remarks were also taken into consideration.

Part II - (λ- and γ-) cyhalothrin			
Version	Date receipt of opinion	Reviewer (only the hub affiliation or European Agency specified)	Comments and short description of changes
Version 2	17.01.2024	Austrian NH	In my view this could benefit from a more thorough ED assessment as this substance has obviously alerts. Please see also the guidance: https://www.efsa.europa.eu/en/efsajournal/pub/5311 According to internal communication (in the frame of biocidal product regulation the substance is currently under assessment) a full ED assessment is warranted! This means that there is an ED concern! We have compiled the information that is currently available. According to this, an endocrine-mediated mode of action is not ruled out. Concern is rather thyroid disruption please see also: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7009157/ https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3138025/ Thank you for the hint. I checked the two quotes above and found no reference to cyhalothrin. ED concern also for the environment: https://pubmed.ncbi.nlm.nih.gov/32806430/ https://www.sciencedirect.com/science/article/abs/pii/S0269749120321345 and also from in vitro studies: https://pubmed.ncbi.nlm.nih.gov/29506456/ Note added Level of confidence in toxicity data should not be "high" given that an ED assessment is ongoing: Assessment Report (including information on DNT and AEL setting): https://www.echa.europa.eu/de/web/guest/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/41/PT18 Assessment done by Greece, full assessment of ED properties is requested Level of confidence downgraded to "medium".
Version 2	17.01.2024	ECHA	Pls note that according to COMMISSION IMPLEMENTING DECISION (EU) 2023/1087, the expire data for the approval of λ-cyhalothrin has been extended until 31 March 2026. Thank you for the hint. Done Pls provide the link to this report: https://www.hbm4eu.eu/wp-content/uploads/2022/07/Pesticides_Substance-report.pdf. Done Could you pls indicate where is it mentioned that the major exposure of the general population to cyhalothrin comes from food residues? Done Toxicological profile: You could include the ECHA references here too. Done Existing evaluations: The EU biocides evaluation is missing. We propose to add it for completeness: Link to Assessment Report 2011: https://echa.europa.eu/documents/10162/6707c237-87db-9346-5b81-c72cc3c35d3f

Part II - (λ- and γ-) cyhalothrin			
Version	Date receipt of opinion	Reviewer (only the hub affiliation or European Agency specified)	Comments and short description of changes
			Link to approval data: https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/factsheet/41/PT18 Added Could you pls indicate the justification for picking this ADI follows below? Added At the time of its approval as biocide, bifenthrin had only one registered product which was a wood preservative authorised in Austria, Denmark, France, Germany, Sweden and the Netherlands. Therefore, the exposure of the EU population to bifenthrin from its biocidal use can be considered indeed very low. Thank you for this information which I added. Have any criteria for characterising the confidence of the HBM-GV been developed under PARC or HBM4EU? Yes, rough criteria have been developed (Apel et al., 2020). The level of confidence (low, medium or high) should reflect the uncertainties identified during the elaboration of the values and could constitute an adequate incentive to later revise values with an estimated 'lower' level of confidence. The levels of confidence are based on expert judgement regarding the accuracy of the data and the process of calculation used to derive the HBM-GVs. Is it possible to indicate what could increase the confidence to "high" level? Considering the complete dataset of cyhalothrin(s) and your conservative rationale for setting the HBM-GV for cyhalothrin, it could be argued that the level of confidence is "high" rather than "medium". Final evaluation of possible ED effects and disclosure of unpublished studies could increase the confidence.
	17.01.2024	EFSA	The HBM-GV is based on the most conservative ADI set in the EU peer review, so the point of departure does not conflict with EFSA's assessment. Ok For the derivation of the HBM-GVs, it appears that the 'second option' approach is followed as described in the paper of Apel et al. (2020; doi 10.1016/j.ijheh.2020.113622). It would be of help to provide a more extensive description of the approach in the report and/or to provide reference(s) to the document that describes the approach. Short description of the different approaches given and reference added. For derivation of the HBM-GV, it is noticed that a 'urinary mass balance approach' has been used. As for this compound, human kinetic datasets are available (references to studies are in the report), providing time-dependent information on plasma and urinary concentrations after oral and dermal λ-cyhalothrin exposure, a PBK model can be made and it would be of interest to assess what HBM-GV would be obtained based on the PBK modelling approach, to better support the setting of the HBM-GV. For personnel reasons, this task can only be carried out in the second part of the project from May 2025. It is also noted that the report does not provide information on how the HBM-GV should be applied. Providing some considerations in the report would be welcome. For example, can the HBM-GV be compared with spot urines? Or

Part II - (λ- and γ-) cyhalothrin			
Version	Date receipt of opinion	Reviewer (only the hub affiliation or European Agency specified)	Comments and short description of changes
			should rather 24-hour urine samples be used? Some general discussion on that can be found in Apel et al. (2020), but it would be help to provide such information also here. Provided in the text: Aylward et al. (2017); Casas et al. (2018)
Version 3	16.12.2024 and 25.02.2025	Adoption by P4.1.3.1.a partners, comments by partners Tamar Berman (MOH-IL), Lucija Perharic (NIJZ) have been taken into account	Monitoring data: It should read 2014 - 2021 (time frame of HBM4EU aligned studies) not 2014 and 2021. Thanks, corrected Discussion, difference between provisional and HBM-GV derived here: This is important explanation but perhaps show differences in Table. Are only differences of the ADI value used? Yes, see Table 5, text slightly modified and reference to Table 5 given Acute Toxicity data on humans: I suggest that the doses are quantified to have an idea what is meant by significant ingestion. Stating the actual toxic doses (if known) would help the understanding of risks to general population due to exposures from food or the environment. I have checked all the cited references again, there was only one quantitative information. I have included this in the text and also deleted "significant", because here it is meaningless. Long term toxicity, human data: I suggest adding the actual doses at which the association or causation was established. In epidemiological studies there is a crucial distinction between an association and causality. It may be more appropriate to explicitly state with which of the effects an association was established and with which a causal relationship. I checked the reference again. Only associations were tested; practically no exposure data were presented that could be used here in the text. Text adapted accordingly.
Final version	1 March 2025		

1.3 Part III - Chromium VI

Part III - Chromium VI			
Version	Date	Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
1	15/4/2024	NH Belgium	Revisions aiming to avoid misunderstandings when referring to the ACSH '*Opinion on limit value setting for non-threshold carcinogens,* especially replacing the term 'acceptable' when referring to the cancer risk level of 4:1000. Other clarifications, e.g. related to workplaces having to reduce the exposure to carcinogens.
1	28/3/2024	NH Germany	Modifications made concerning the terminology used related to the different Cr(VI) species workers can be exposed to, and their solubility.
1	10/04/2024	NH France	Further information provided on e.g. current occupational exposure levels.
1	24/03/2024	NH Sweden	Confidence levels have been re-evaluated, their choice has been elaborated, and a reference to the criteria used has been made clearer. Further discussion and information included (e.g. elaboration on why the focus was not to derive an HBM-EECR for RBC-Cr, information on RPE-usage in Table 1).
1	12/04/2024	NH Poland	No revisions were proposed
1	11/04/2024	Partner Maria Tannous and Fatoumata Sissoko, ANSES, France	Confidence levels have been re-evaluated. Further detail added on several aspects, including the information given in table 1 and derivation of risk levels.
2		Adoption by P4.1.3.1.a partners	
Final Version	01/03/2025		

1.4 Part IV - Nickel

Part IV - Nickel			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
		Jonna Weisell-Laitinen	First draft
Version1	24.11.2023	ANSES/Maria Tannous and Keyvin Darney	Chapter about PBPK modelling added and PBPK modelling considered in derivation of HBM-GV general population. Proposal of HBM-GV general population changed to more conservative.
Version1	24.11.2023	NIJZ/Manca Ahačič and Lucija Perharič	Complement of the glossary, Equations clarified.
Version 2	24.04.2024	German sub-hub human tox	Uncertainties to HBM-GV for general population were added. Due to uncertainty between the uptake of nickel in food and available data on nickel sulfate, it was abstained to derive HBM-GV. Instead, orientation HBM range was proposed.
Version 2	15.4.2024	German sub-hub human tox	Clarifications on recommendations made by DFG. Correlation lines between workers urine nickel concentration and air nickel concentration were added. References were added.
Version 3	12.11.2024 and 29.11.2024	Adoption by P4.1.3.1.a partners, comments considered from Tamar Berman (MOH-IL), Manca Ahačič (NIJZ)	Updated table of general population urine nickel concentration. Clarified that nickel has been shown to cause occupational asthma. Skin sensitization chapter moved after acute toxicity. Information about systematic contact dermatitis added including EFSA evaluation. Use of systematic contact dermatitis was considered if it could be used as a basis for HBM-GV derivation. However, it was concluded that systematic contact dermatitis could not be used as a starting point of HBM-GV derivation. ATSDR 2024 minimal risk levels were included and suitability for HBM-GV starting point was considered. Definition of orientation HBM-GV range was added. Discussion has been made more in line with the definition of orientation HBM-GV range.
Final Version	03.03.2025		

1.5 Part V - DEHTP

Part V - DEHTP			
Version	Date	Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
1	19/03/2024	UBA	First compiled version for review of PARC partners
2	07/06/2024	Comments from: Partners Manca Ahacic/NIJZ, Maria Tannous/ANSES, Keyvin Darney/ANSES Experts from NH Austria, NH Belgium, NH Swiss	Revised version, comments considered which were provided by reviewers. Comment: Use L for liter instead of l. Done throughout the text **Comment on literature search:** PRISMA diagram would be relevant here. Added. Additional information: If the original source was not available and the information was only taken from reports, this is noted in the tables in the 'named in' column Comment: I would suggest to state in one of the chapters, that DEHTP is not harmonised classified according to CLP (ECHA, 2023). Done, see 3. Further, I would also suggest to state that it is restricted in FCMs (please check). DEHTP is allowed to be used in food contact materials **Comment on 3.3:** Please add reference. Done **Comment regarding exposure/ content of DEHTP in products:** Certainly not true since it is in >10% in some products. Statement deleted Comment: Maybe worth mentioning the toxicological endpoint on which this TDI was based? Added **Comment on 5.1.1 and also later in the text:** Add a description of existing models; Development, testing, parameterisation, and calibration of a human PBPK model for the plasticiser, di-(2-ethylhexyl) terephthalate (DEHTP) using in silico, in vitro and human biomonitoring data; https://doi.org/10.3389/fphar.2023.1140852. Then you will have to justify the choice of a mass conservation approach over PBK. Points adressed Comment: Was the rest excreted as DEHTP unchanged? Answer: Only urinary excretion was considered, therefore no statement can be made in this regard **Comment on 5.1.3:** In childbearing age? Answer: Yes **Comment on 5.2.1:** Suggestion to also mention the study on the percutaneous absorption rate mentioned at the beginning of the report: percutaneous absorption rate of 0.103 ± 0.052 µg/cm²/hr. Answer: Text adapted accordingly Comment: If this is referring to the study by Guerin et al., 2002, it would be better to have them together. Answer: Text adapted accordingly

Part V - DEHTP			
Version	Date	Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
			Comment on 5.2.3, Studies with repeated exposure: Suggestion to further describe this study here as it is the key study for the derivation of EFSA's and ECHA's reference values. Please expand description of the study, species, sex, number, exposure type, doses, etc. so you can later develop the derivation of EFSA's TDI. Done **Comment on 6.1:** After reading this chapter we do not know what is the critical effect. Effects named Comment: Mortality is not relevant when it comes to critical effect, even more when you are looking at internal levels. Answer: It is just an introduction into the toxicity Comment: Study description should come sooner in the toxicological profile. Then you should already have enough information from chapter 5 to directly state the critical effect, identify the key study and the PoD. Answer: adapted accordingly Comment: For phthalates, DMP is less toxic than DEHP…Not sure if there is a TDI for DMP… (to have a comparison with DEHP). For DEHTP vs DMTP, having similar TDI seems rather strange. Answer: Text passage deleted because misleading Comment: Please make it clear whether RAC assessment is available or not. Answer: RAC assessment is not available, text adapted accordingly Several similar comments: They used the same key study and used a BMD approach. Actually, I would have more confidence in this approach rather than the NOAEL/LOAEL approach. Comment: A better reason to choose TDI over DNEL would be the choice of UFs, which should be discussed. ECHA's choice of UF is actually less protective and there seems no reason to lower default UF from 10 to 4 and 5 for UF_A and UF_H respectively. You could have refine UF_A to 2.5 using allometric scaling or PBK modelling for instance. Answer: Now we have used the $BMDL_{10}$ related to effects on the retina and EFSA standard assessment factors Comment: Define HBM-I. Done Comment: Please add reference for the Fue values and expend so we can judge of their relevance. Answer: Fue are now explained above Comment: Very clear. Thank you for giving equation's details. You can improve readability by putting out the units and instead define terms after Eq.1 and Eq.2. Answer: We would prefer to keep the units as this is the scientific correct way. Comment: If not a TDI either define or just give the value as TDI-like does not give any information. Answer: Text has been reworded Comment: Some details given are not relevant for a BMD, what are the BMD and the BMDU values? Answer: Text has been reworded, BMD is given now

Part V - DEHTP			
Version	Date	Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
			Some similar comments: Develop why an effect or assessment value is not relevant. Answer: Reasons explained Some comment: Not relevant here. Actually, they had rounded up the TDI to 1 because of the many uncertainties in its derivation. This would be a reason to diminish confidence in the HBM-GV value. Answer: TDI not used anymore, $BMDL_{10}$ related to effects on the retina has been used now. Comment: You cannot be confident when the values are from only one study, three males and one dose. Answer: This is exactly what is mentioned in the sentence after Comment: TK variability is covered by default and DEHTP specificities are not taken into account due to a lack of data, this decreases confidence level. Answer: Text adapted accordingly Comment: TK variability covered to some extent, not fully. Answer: Text adapted accordingly Comment: I would suggest to note this also above in the previous chapters, where the results of Lessmann (2016) are presented. Answer: Text adapted accordingly Comment: Good point. Maybe you can specify what percentage of the concentrations seen after dosing was represented by the "background" concentration. Eventually to prove that the background was irrelevant in this case. Answer: Text adapted accordingly Comment: I would consider it medium at best. Limited number of chronic studies, only one TK study to assess the Fue, default UF. Answer: Adapted accordingly, LoC classified as “medium” List of acronyms expanded and editorial changes made acc. to comments of the reviewers
3	24.02.2025	Final approval by the P4.1.3.1.a partners. No further comments	
Final version	01.03.2025		

1.6 PART VI - DINP

PART VI - DINP			
Version	Date	Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
1	30.05.2024 07.06.2024 07.06.2024 07.06.2024	Experts nominated by UK NH, Austrian NH Norwegian NH Slovenian NH	Names/descriptions of individual isomers have been corrected. Information on classification according to CLP has been added. Information on exposure has been specified. Level of confidence has been attributed to individual considerations. The following note has been added (chapter 6.4): It is known that in some analysis methods, OH-MINP might have an interference from a carboxyl metabolite of terephthalate if they are not separated. And this may give wrong results for urinary concentrations of OH-MINP. A reference to an existing provisional HBM-GV for workers and information on its derivation has also been included. The future revision of this value under PARC has been announced. Abbreviations have been added and editorial revisions have been made.
2	17.02.2025	Approval by P4.1.3.1.a partners	No further comments
Final version	17.02.2025		

1.7 PART VII - Aluminium

PART VII - Aluminium			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
Draft version	21/06/2024	ANSES	First report version shared for consultation
Consultation phase (National Hubs and project partners)	23/07/2024	German Federal Institute for Risk Assessment BFR	Wording correction. Suggestion to add HR-ICP method in section 5.1 as well as the G-EQUAS extern quality assurance scheme. Clarifications regarding the derivation of the BAT value. Suggestion to consider the meta-analysis done by the DFG-BAT working group for the derivation of HBM-GVs.
	15/08/2024	German Environment Agency, UBA	Wording correction. Suggestions and guidance to develop the abstract. Request to explicitly explain why the Biomonitoring Equivalents were not retained. Suggestion to also add volume-based HBM-GVs (in µg/L).
	05/09/2024	Finnish Institute of Occupational Health	Suggestion to add more discussion on the toxicokinetic differences of soluble and insoluble/sparingly soluble aluminium compounds, and the impact of these differences in the applicability of the proposed HBM-GVs.
	05/09/2024	UK Health Security Agency	Suggestion to add confidence level to the derived HBM-GVs.
	09/09/2024	National Institute of Public Health of the Republic of Slovenia	Rewording suggestions. Request to add precision to some abbreviations. Suggestion to add in section 4.13 more details on the sensitive population (exposure of patients with renal failure to aluminium via phosphate binders, antacids and aluminium-containing dialysis fluids).
	17/09/2024	Institute of Environmental Medicine, Sweden	Suggestion to add information on some effects (sensitisation, dialysis encephalopathy, restrictive pulmonary disease, obstructive pulmonary disease, cardiovascular disease, haematological effects, bone related effects, cardiovascular disease) and the existing guidelines. Suggestion to add aluminium phosphide in the scope of research (section 3.1).

PART VII - Aluminium			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
Revised version	23/10/2024	ANSES	-Formatting and rewording suggestions were considered when convenient. -Authors have added an abstract to the report. -Aluminium phosphide has not been added to section 3.1 as the toxicity of this compound mainly involves phosphine gas and is not related to aluminium. -An addition has been made to section 4.1.1 explaining that the applicability of HBM-GVs is not affected by whether the initial form of aluminium is soluble or insoluble. -Suggested information were added to the chapter on sensitisation (4.6.1). -A brief summary of dialysis encephalopathy has been included in the chapter on chronic neurotoxicity. -The description of restrictive and obstructive lung diseases has not been expanded, since in section 4.8.2.1 these effects are mentioned but not detailed, due to co-exposures to other toxic chemicals, in particular irritants. -Chapters on haematological and bone related effects have been developed on the basis of suggestions (see sections 4.8.3 and 4.8.4) and the slightly increase cardiovascular mortality in miners was mentioned in section 4.8.5 with a precaution of interpretation. -Details on the exposure of renal failure patients to aluminium have not been added to section 4.13 but some information was integrated in section 4.8.1 'dialysis encephalopathy'. -The G-EQUAS external quality scheme was listed among the others in section 5.1 and HR ICP-MS was also mentioned. However, this analytical technique was not very discussed in details as it is very costly. -The existing guidelines on aluminium were not added to avoid making the document more cumbersome and given that this part is not usually included in HBM reports. -In chapter 7.2, a question related to the study by Letzel et al. (2000) was raised, mainly as to whether the difference in results from Buchta and Kiesswetter's findings could be explained by the different aluminium species present in aluminium powder plants and in the car body construction industry. However, we believe that exposure and bioavailability are reflected by the concentration of aluminium in urine that was compared, and that this parameter was considered when evaluating the studies. One hypothesis could be the possible contamination of urine samples. -A reasoning paragraph explaining why biomonitoring equivalents were not retained has been added to section 7.4. -Clarifications and corrections have been made in section 8 concerning the BAT value.

PART VII - Aluminium			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
			-It was preferred to use Buchta et al. (2003) and Kiesswetter et al. (2009) as key studies and not base the HBM-GV on the meta-analysis carried out by the DFG-BAT working group, because some of the studies considered in this meta-analysis were not retained in our assessment. -The volume-based HBM-GVs were not added because an adjustment was deemed necessary to account for the high concentration variability, particularly due to water intake (very dilute or highly concentrated urine). -An additional section (10) was added to the report, assigning a medium-high confidence level for the derivation of the two HBM-GVs.
Revised version by ANSES	23/10/2024	For approval by P4.1.3.1.a partners	No further comments
Final version	31/01/2025		

1.8 Part VIII - Benzophenone

Part VIII - Benzophenone			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
Version 1	02.07.2024	UBA	First consolidated version for review by PARC partners and national hubs
Version 1		Partners Manca Ahačič (NIJZ), Lucija Perharič (NIJZ), Maria Tannous (ANSES) Christophe Rousselle (ANSES) Experts from Danish NH Belgian NH Norwegian NH UK NH	Clarified that only HBM-GV for the general population are derived. Keywords, abbreviations, and references added. Reason for the reference of the HBM-GV to urine volume clarified and stated later in the dossier. Defaults for the urine volume specified Distinction made between primary and secondary sources of BP-3 exposure. Background to regulation clarified and updated. Clarification that the classification is a self-classification. Figure on biotransformation pathway added. Clarification, where necessary, that the measurement of deconjugated BP-3 refers to the measurement of total (conjugated and free) BP-3 Precision of study data (dose converted from ppm in feed, matrix studied, measurement of total BP-3 or free BP-3, more precise description of effects)
Version 2	09.03.2025	For approval by P4.1.3.1.a partners Comments by Tamar Berman considered	Link to VITO dashboard and reference Govarts et al., 2023 added
Final Version	12.03.2025		

1.9 Part IX - Acetamiprid

Part IX - Acetamiprid			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
1	02/07/2024	UBA	First compiled version for review
1	02. – 05.09. 2024	Partners: Manca Ahačič (NIJZ), Lucija Perharič (NIJZ), Paul Scheepers (Radboudumc) Experts from Belgian NH UK NH	There should be also attention for the analytical methods. Short paragraph inserted. There should be a facsheet at the end. As well as a deliberation and overview on the 'level of confidence' of various aspects etcetera. Factsheet added, level of confidence summarized and not listed again separately to avoid repetition. Is there a reason to use so many abbreviations for thiacloprid and thiamethoxam? This is just due to the fact that authors use different abbreviations and we kept them in the Annex tables. What about faecal excretion? Added that no information on faecal excretion is available for the relevant metabolism studies, as the focus here was on urine. However, see the tables for further information. Add a figure on metabolim. Done Harada: Of volunteers how many were male and how many female? Information was not given in the publication Is the molar fraction of the parent expressed as % of the dose? Not clearly stated but it is assumed to be the molar fraction. 2024 EFSA document should be considered. Yes, done. Further textual clarifications have been made and the list of abbreviations has also been supplemented.
2	11.03.2025	For approval by P4.1.3.1.a partners Comments from Tamar Berman considered	Standardisation of abstract and results on HBM-GV derivation regarding underlying EFSA TDIs from 2013 and 2024

Part IX - Acetamiprid			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
Final version	14.03.2025		

1.10 Part X – Imidacloprid

Part X – Imidacloprid			
Version	Date	Reviewer name/Institutions	Short description of changes
1	15/11/2024		First compiled version for review by PARC P4.1.3.1.a partners
2	08/01/2024	ANSES, MOH-IL, TTL	Second version after commenting of PARC P 4.1.3.1.a partners
3	12/03/2025	Comments from Austrian NH, German NH, Spanish NH, Swiss NH, UK NH	Revised version, comments considered as far as possible. General: Terms and headings clarified and additional information on tests added. Introduction: more information needed regarding potential human health effects reported in the literature, definition of the HBM-GVs and why they are needed, main objectives of this report. Information added. nAChRs of insects? This might be important although nAChRs are present in both (insect and mammalian) nervous systems. Yes, of insects, text modified. Literature search strategy and exclusion criteria revised and explained in more detail. Insecticide category and veterinary application supplemented. The general population is mainly exposed through food and drinkable water. For the occupational population, including farmers, their families if they apply pesticides or biocides, the workers, etc., the primary route of exposure is skin (90%) and inhalation (10%). Yes, text reformulated. In the latest EFSA report (https://multimedia.efsa.europa.eu/pesticides-report-2021/) on residues in food, imidacloprid appears in 3 samples, although the MRLs are not exceeded. Information added. Be more specific or explain what «relevant institutions» are. Done The metabolism chapter, including the rationale for the Fue, has been completely revised in line with the comments. What about poisoning? E.g. https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2024.1421437/full https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2024.1421437/full#B18

			https://pmc.ncbi.nlm.nih.gov/articles/PMC5699009/ https://www.researchgate.net/publication/259169805_Accidental_human_poisoning_with_a_neonicotinoid_insecticide_imidacloprid_A_rare_case_report_from_rural_India_with_a_brief_review_of_literature Poisoning is discussed in a subchapter, and the studies found are summarized in the Appendix, Table 7. The text has been modified for clarity. It would be even better to list the type of epidemiological studies (i.e., cohort study, observational, case-control, etc.) and to add for each type the number of studies. Done Case control studies: Specify who were the controls. Done Describe limitations of studies in more detail and justify why the studies described are not suitable for HBM-GV derivation. Done Details of animal studies were given as possible from information source. Many studies could not be viewed in the original because they were company studies. Therefore, the level of confidence was corrected downwards to low-medium. Nevertheless, the assessment was based on critical evaluations previously done by (mainly) authorities so that it can be assumed that a reliable assessment was carried out (see also Appendix, Table 8 ff). Differences in methodology or in study design or methodological shortcomings? Adapted accordingly Selection of the key study better justified. We know that oxidative stress is one of the early markers in several AOPs, therefore it should be mentioned here. Added Please see: https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/imidasumm.pdf Subchronic Non-Cancer Health Risk Limits(nHRLSubchronic) = nHRLShort-term = 2 µg/L Please see also: https://www.intechopen.com/chapters/79254 (effects in humans, neurotox described among others) Considering the 1) Persistency in the environment 2) Chronic neurotoxic effects in animals and humans. The subchronic non-cancer health risk limit is based on the POD value of 0.820 mg/kg/d, which is still higher than the POD used in our assessment. Neurotoxicity is already described in the document here and most of the studies (relevant for humans) cited in the provided reference are also cited in the report. The provided link summarizes also effects related to ecotoxicity, which is out of scope for this work. The last paragraph of the publication (second link provided in the comment) summarizing effects related to humans is mainly about chlorpyrifos and not IMI. Precise the conditions for using the option 1, 2 or 3 to derive an HBM-GV. Done. See also reference to the strategy paper.

			Including a summary table with the values of key parameters (ADI, Fue, urine rate, MWs) along with the resulting HBM-GVs could make the information more visually accessible. Included Level of confidence (LoC): Two statements saying medium is ok, one statement saying should be low. LoC lowered to low - medium
Final version	April 2025		

1.11 Part XIa - Inorganic and elemental mercury

Part XIa - Inorganic and elemental mercury			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
Version 1		Jonna Weisell-Laitinen	First compiled version for review by PARC P4.1.3.1.a partners
Version 2	25.01.2025	Jonna Weisell-Laitinen	Draft version considering comments provided by partners
	28.2.2025	NH Spain	Editorial improvements have been made. A table including justification of an assessment factor for the general population was added. More detailed explanation and justification of a HBM-GV derivation for the general population was added including equations for unit conversion and justification of the assessment factor. No changes were made in the AF as most of the commenters agreed the value and opposite comment were given. Confidence level was changed to medium due to uncertainties.
	28.2.2025	NH Spain	It was noted that NOAEL and LOAEL terms were used incorrectly with the biomonitoring. However, these terms have been used before in similar kind of tables to show critical value (e.g. in the cadmium deliverable in the HBM4EU project). Also, it was considered that these terms are well known rather than invent some new values for this purpose. Text has been clarified and details added for multiple parts especially in regulation of the lamps, occupational exposure, and exposure through dental amalgams. Editorial improvements have been made. Table 3 was rearranged and subheadings were added to separate external and internal guidance values. It was commented that newer references should be added. However, we have identified all the relevant studies, and no newer original studies were identified that could be useful for the dose-response assessment.
	28.2.2025	NH Spain	It was noted that NOAEL and LOAEL terms were used incorrectly with the biomonitoring. However, these terms have been used before in similar kind of tables to show critical value (e.g. in the cadmium deliverable in the HBM4EU project). Also, it was considered that these terms are well known rather than invent some new values for this purpose.

Part XIa - Inorganic and elemental mercury			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
			It was suggested to add information about cumulative effect and long-term effect of HBM-GVs measured in urine. However, this was not added as this is not applied for all the cases. This depends on the substance in which matrix it is detected and whether substance is cumulative. Explanation of the exposure of dental amalgams even when placed was clarified. Relation of proposed HBM-GV and BAT value is added in the discussion section. It was commented that difference in HBM-GV between the general population and workers is too high. However, it is only 2-fold and some commenters were in the opinion that the difference is too small to account for the variability among the general population. It was commented that newer references should be added. However, we have identified all the relevant studies, and no newer original studies were identified that could be useful for the dose-response assessment.
	14.2.2025	NH Poland	Reference of the ATSDR report was changed to the final version from the draft version. At the same time, it was confirmed that no changes in the MRL calculations were made since the draft report. It was noted that for the general population there is lack of data to fully use the approach that links urinary mercury concentrations to health effects as the epidemiological studies in the general population are limited. This note was considered to lower the confidence level of the HBM-GV from high to medium. Future aspects were added in the discussion.
	26.2.2025	Partner: Lucija Perharič (NIJZ),	Explanation on calculations of cumulative mean urinary concentration and its significance was added.
	26.2.2025	NH UK	Corrected language and typos. Confidence level was changed to medium due to uncertainties. Equations of the unit conversions added. More detailed explanation and justification of a HBM-GV derivation was added including equations for unit conversion and justification of the assessment factor. Hyperlinks to the table 3 was added.

Part XIa - Inorganic and elemental mercury			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
			It was commented as there were presented quite many studies non key studies could have been removed. As we got some opposite comments as well, thus, all the studies were left in the deliverable. However, we rearranged the order to better identify the key studies and supporting studies. It was suggested to add summary table in the end of the deliverable. However, there is already the fact sheet in the end of the report this was not considered necessary, thus, not added.
	28.2.2025	NH Finland	Considered well justified. No further comments or requests were made.
	28.2.2025	NH Switzerland	General information of the project and aims were added in the abstract. In addition, more details of the general population HBM-GV derivation was updated. However, no changes made in the abstract part of the occupational HBM-GV derivation. The data is presented in detail in the main text. It is not possible to reach as big numbers in occupational studies as in general population studies. Considering that were several studies from different countries pointing all to the same direction we consider the database to give a good reflection of the situation. In addition, we want to emphasise that in these studies the exposure characterization and examination of workers have been more detailed than in larger epidemiological. These aspects have been discussed in the main text. Since other reviewers did not see need to increase the intraspecies AF, either, we will retain it as 1. Aims of the project have been better emphasized. Editorial changes were made to improve language, clarity and consistency. Neurotoxicity studies were rearranged to better identify the key studies and supporting studies. Limitation of the sex distribution in the occupational studies was added in the study limitations. Table 1 and 2 were made to easily compare critical value between the studies, thus, not all cohort details were added in the table. It was suggested to consider the fact that in some of the occupational studies no urine measurements were collected from the controls as a relevant data gap. We agree that this is a limitation but if the selection of controls was done carefully, ensuring by other means (e.g. questionnaires) that the controls had no occupational or recreational exposure to Hg, we do not consider this a significant limitation. More information about reproductive and developmental toxicity was added.

Part XIa - Inorganic and elemental mercury			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
	10.3.2025	NH Greece	Considered well justified. No further comments or requests were made.
Version 3 considering comments by NH experts and partners	11.03.2025	For approval by P4.1.3.1.a partners	
Final version	14.03.2025		

1.12 Part XIb - Methylmercury

Part XIb - Methylmercury			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
version 1	17/01/2025	Helle Katrine Knutsen	First compiled version for review by PARC 4.1.3.1.a partners.
version 2	25/01/2025	Helle Katrine Knutsen	Revised version considering comments provided by PARC partners
Version 2	28/02/2025	Polish NH	The value of the EFSA TWI expressed as a daily dose (0.188 µg Hg/kg bw) was added. It was a suggestion to explain that the HBM-GV might need update based on new evidence. This was not included since it applies to all HBM-GVs.
Version 2	28/02/2025	Finnish NH	Considered well justified, no changes made.
Version 2	28/02/2025	UK NH, Emily Bird	TWI and UF defined at first appearance in the abstract. Hyperlink to HBM4EU WP13 D13.7 was added for easier access to figure 5 in that report.
Version 2	28/02/2025	Spanish NH JV Tarazona	Hyperlink to HBM4EU WP13 D13.7. was added. The reviewer suggested to harmonize the terms Reference point (RP) and point of departure (POD) in part a (inorganic HG) and b (MeHg). This was not done since the HBM-GV for MeHg was based on EFSA's risk assessment from 2012. Changing this could lead to confusion. RP was added to the PARC glossary. The term "expressed as mercury" was removed as suggested by the reviewer and replaced by Hg/volume or mass. Also, Hg was added at places where only µg/kg was initially indicated. "Range of 1.8 mg/g hair" has been replaced by at "approximately 1.8 mg Hg/kg". The reviewer suggested to explain the link between approximately 1.8 mg Hg/kg (in the introduction) and hair concentrations up to ~4.0 mg/kg. This could not be explained, since there is no direct link. Editorials addressed

Part XIb - Methylmercury			
Version	Date	The person(s) who prepared/reviewed the version Reviewer (only the hub affiliation or European Agency specified)	Short description of changes
Version 2	28/02/2025	Spanish NH Pablo Olmedo Palma	Editorials addressed
Version 2	28/02/2025	Spanish NH Fernando Gil Hernanández	The reviewer suggested reviewing 4 concrete papers on Hg exposure to cover the period 2023 to 2025. Since these papers did not address risk of adverse effects from Hg exposure, they were not further considered.
Version 2	28/02/2025	Slovenian NH Lu Perharic	Editorials addressed
Version 2	28/02/2025	Swiss NH Joyce de Paula Souza	Abstract was expanded Conclusion chapter added
Version 2	10/03/2025	Greek NH	The reviewer indicated that the derived HBM-GVs could underestimate the risk of MeHg and listed several reasons, such as difficulties in measuring behavioral and cognitive changes, interindividual variability, lack of conclusive evidence for ADHD-related endpoints and lack of regulatory acceptance of cardiotoxicity. The reviewer indicated a need for an extra UF to account for such factors. This was not deemed necessary given the broad agreement from other reviewers, and if future evidence indicate a need, the HBM-GVs can be updated.
Version 3	05/03/2025	For approval by P4.1.3.1.a partners	
Final version	12/03/2025		