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First report on the development and validation of the integrative models

WP 8 – T8.3



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Lead Beneficiary/ Responsible AE	WR-BIOM (NL), AUTH(EL)
Contributing Participants	ANSES (FR), INERIS (FR), VITO (BE), ISSeP (BE), MU (CZ), DTU (DK), SYKE (FI), TTL (FI), IRFM (IT), IUSS (IT), UL-LACDR (NL), WU-TOX (NL), NIVA (NO), IISPV (ES), INSST (ES), NIC (SI), UU (SE), UOC (EL), UT (EE)
Responsible author(s)	Jasper Engel / WR-BIOM / jasper.engel@wur.nl Johannes Kruisselbrink / WR-BIOM / johannes.kruisselbrink@wur.nl Spyros Karakitsios / AUTH / spyrosk@auth.gr Denis Sarigiannis / AUTH / sarigiannis@auth.gr
Co-authors	Amélie Crépet / ANSES / amelie.crepet@anses.fr Madeline Carsique / ANSES / madeline.carsique@anses.fr Clément Blassiau / ANSES / clement.blassiau@anses.fr Philippe Palmont / ANSES / philippe.palmont@anses.fr Achilleas Karakoltzidis / AUTH / karakoltzidis.achilleas@gmail.com Nafsika Papaioannou / AUTH / nafsikpa@office365.auth.gr Dayna Schultz / AUTH / daynaraeschultz@gmail.com Deepika Deepika / IISPV / deepika@iispv.cat Vikas Kumar / IISPV / vikas.kumar@urv.cat Aude Ratier / INERIS / aude.ratier@ineris.fr Emilio Benfenati / IRFMN / Emilio.benfenati@marionegri.it Gianluca Selvestrel / IRFMN / gianluca.selvestrel@margionegri.it Jiří Kalina / MU / kalina@mail.muni.cz Klara Komprdová / MU / klara.komprdova@recetox.muni.cz Daria Sapunova / MU / dario.sapunova@recetox.muni.cz Knut Erik Tollefsen / NIVA / knut.erik.tollefsen@niva.no Sam Welch / NIVA / sam.welch@niva.no Walter Zobl / NIVA / walter.zobl@niva.no Viviane Girardin NIVA / viviane.girardin@niva.no Arno Vanderbeke / VITO / arno.vanderbeke@vito.be Katleen de Brouwere / VITO / katleen.debrouwere@vito.be Ola Spjuth / UU / ola.spjuth@uu.se

Internal Reviewers ²	Name of the Internal Reviewer(s) / short name of institutions/email addresses Denis Sarigiannis / AUTH / sarigiannis@auth.gr
External Reviewers ³	Name of the External Reviewer(s) / short name of institutions/email addresses Matthias Herzler / BfR/ matthias.herzler@bfr.bund.de Thomas Russell / EPA / Thomas.Russell@epa.gov Jean-Lou Dorne / EFSA / jean-Lou.dorne@efsa.europa.eu
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¹ PU = Public

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Abstract

This report provides an update on the activities within PARC Task 8.3, which focuses on developing a digital ecosystem or network that links data, models and tools for integrative chemical risk assessment. The ecosystem is designed and applied in practical case studies in collaboration with partners across PARC WP5, WP6 and WP7.

A bottom-up strategy is being used to shape the ecosystem, with a primary focus on developing and validating workflows that connect specific modelling tools within case study contexts. This report illustrates how this approach is establishing a network, where key modelling platforms – INTEGRA, MCRA and STOP – act as central hubs, linking various modelling tools and data sources while facilitating access to workflows. The adoption of an updated overarching conceptual framework provides a top-down perspective, clarifying the roles and interconnections of the modelling tools and workflows within the network.

The workflows presented collectively address key aspects of chemical risk assessment as captured in the conceptual framework. These include aggregate (cumulative) risk assessments based on modelled or measured exposures, estimation of attributable disease burden, and the integration of new approach methodologies. Workflows are applicable to human or environmental effects. Given that they are at different stages of development, their validation ranges from proof-of-concept demonstrations to external model validation and broader acceptance testing.

Key Words

Harmonisation; Workflows; Software; Data analysis; Models; Uncertainty assessment; Integrated risk assessment; Human risk assessment; Environmental risk assessment; FAIR

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Acronyms

AD: Applicability Domain
AEA: Aggregate Exposure Assessment
AEP: Aggregate Exposure Pathway
qAEP: Quantitative Aggregate Exposure Pathway
AO: Adverse Outcome
AOP: Adverse Outcome Pathway
AOPN: Adverse Outcome Pathway Network
BoD: Burden of Disease
CERAPP: Collaborative Estrogen Receptor Activity Prediction Project
COMPARA: Collaborative Modeling Project for Androgen Receptor Activity
DR: Dose Response
EBD: Environmental Burden of Disease
EDI: Estimated Daily Intake
eFAST: Extended Fourier Amplitude Sensitivity Test
FAIR: Findable, Accessible, Interoperable, and Reusable
GDPR: General Data Protection Regulation
HBM: Human BioMonitoring
HBMGV: Human BioMonitoring Guidance Value
HI: Hazard Index
HQ: Hazard Quotient
HC: Hazard Characterisation
IRA: Integrative Risk Assessment
KE: Key Event
MC: Monte Carlo
MCMC: Markov Chain Monte Carlo
MH-MCMC: Metropolis Hastings MCMC
MCRA: Monte Carlo Risk Assessment toolbox
MIE: Molecular Initiating Event
MRA: Mixture Risk Assessment
mRPI: Modified Reference Point Index

NAM: New Approach Methodologies
NOEC: No Observed Effect Concentration
NSEC: No Significant Effect Concentration
NRMEA: Nuclear Receptor-Mediated Endocrine Activity
OR: Odds Ratio
PAF: Population Attributable Fraction
PBK: Physiologically Based Kinetic
PCP: Personal Care Product
PFAS: Per- and Polyfluorinated Alkyl Substances
PFOA: PerFluoroOctanoic Acid
PFOS: PerFluoroOctaneSulfonic Acid
PNEC: Predicted No Effect Concentrations
PoD: Point of Departure
PPP: Plant Protection Product
MOET: Margin Of Exposure Total
QMRF: QSAR Models Reporting Format
QSAR: Quantitative Structure-Activity Relationship
QSPR: Quantitative Structure-Property Relationship
RA: Risk Assessment
RAdb: NIVA's Risk Assessment Database
RPI: Reference Point Index
RPF: Relative Potency Factor
RR: Relative Risk
RQ: Risk Quotient
SBML: Systems Biology Markup Language
SMILES: Simplified Molecular Input Line System
SRQ: Sum of Risk Quotients
SSD: Species Sensitivity Distribution
STOP: Source To Outcome Pathway
TD: Toxicodynamic
TK: Toxicokinetic
UI: User Interface

1 Introduction

Integrative risk assessment (IRA) plays a key role in understanding the health and environmental impacts of chemical exposure. By combining data and models from multiple disciplines such as exposure science, epidemiology, toxicology and risk assessment (RA), integrative approaches may enable for instance a more comprehensive understanding of chemical risks, more accurate assessments, and more robust and informed decision-making. Examples of such assessments include modelling exposures from multiple sources and routes, the integration of measured exposures from human biomonitoring (HBM) studies, and assessments that make use of new approach methodologies (NAMs) such as chemical grouping based on -omics data or quantitative structure-activity relationship (QSAR) modelling.

The practical implementation and operationalisation of IRA requires workflows that effectively harmonise and connect diverse modelling tools and datasets. However, this process is often hindered by a lack of alignment, as tools and data are typically developed independently, using varying concepts, data standards and programming languages. PARC T8.3 seeks to bridge this gap in interoperability, aiming to establish a network of interoperable tools and datasets that enables their seamless use in IRA.

The development of this network is iterative. Earlier work, in particular PARC deliverable 8.3 and Additional Deliverable 8.4, presented the initial conceptual and technical design of the network, illustrating how it could link models across the continuum from chemical release into the environment to (human and environmental) exposure and the resulting (health) impact [1, 2]. Additionally, these Deliverables highlighted preliminary findings from the application of several workflows within the model network in (PARC) use cases.

This Deliverable builds upon this foundation by presenting and demonstrating the workflows that are currently available in the PARC model network. The workflows cover a wide range of assessments such as HBM-based (mixture) RA, aggregate exposure assessment (AEA), environmental burden of disease (EBD) assessment, and chemical grouping. By describing the workflows in a harmonised way, using an updated conceptual framework, it is shown how they relate to one another, illustrating the potential for workflows to be linked sequentially or for specific components to be reused across different workflows. In particular, the reuse of specific model classes (such as PBK models) and propagation of uncertainties throughout workflows is discussed.

2 The PARC model network

The PARC model network (<https://www.parc-models.eu/>) is a digital ecosystem designed to provide researchers, regulators and other stakeholders in Europe with access to a wide range of modelling tools and workflows for addressing questions about the (health) effects of chemical exposure. The network aims to:

- Support harmonised, scalable, and cross-sector IRAs across Europe, aligning with
 - a. regulatory requirements (e.g. General Data Protection Regulation (GDPR) and transparency regulations),
 - b. international guidance (e.g. on transparent publication of model code, see OECD [3, 4]), and
 - c. regulatory methodologies (e.g. from EFSA [5]) as well as novel methodologies from PARC.
- Develop and operationalise user-friendly and generic workflows that potentially link multiple domains, modelling tools and datasets to provide solutions for complex IRA questions.
- Offer a structured overview of selected modelling tools and workflows suitable for answering IRA questions.
- Enhance interoperability and reusability of selected modelling tools to facilitate their efficient and practical implementation in integrative assessments.

2.1 Conceptual framework

As previously described, a conceptual framework forms the basis of the model network [1, 2]. It is designed to characterise modelling tools from (potentially) diverse domains, identify relationships between these tools, and describe workflows. The framework is being developed iteratively. In its initial iterations it described integrative assessments at a high level without distinguishing specific data inputs and modelling steps within workflows [1]. A subsequent refinement made the framework applicable to selected workflows [2]. Figure 1 presents the latest version of the framework, which now captures all modelling activities relevant for the workflows in the present Deliverable.

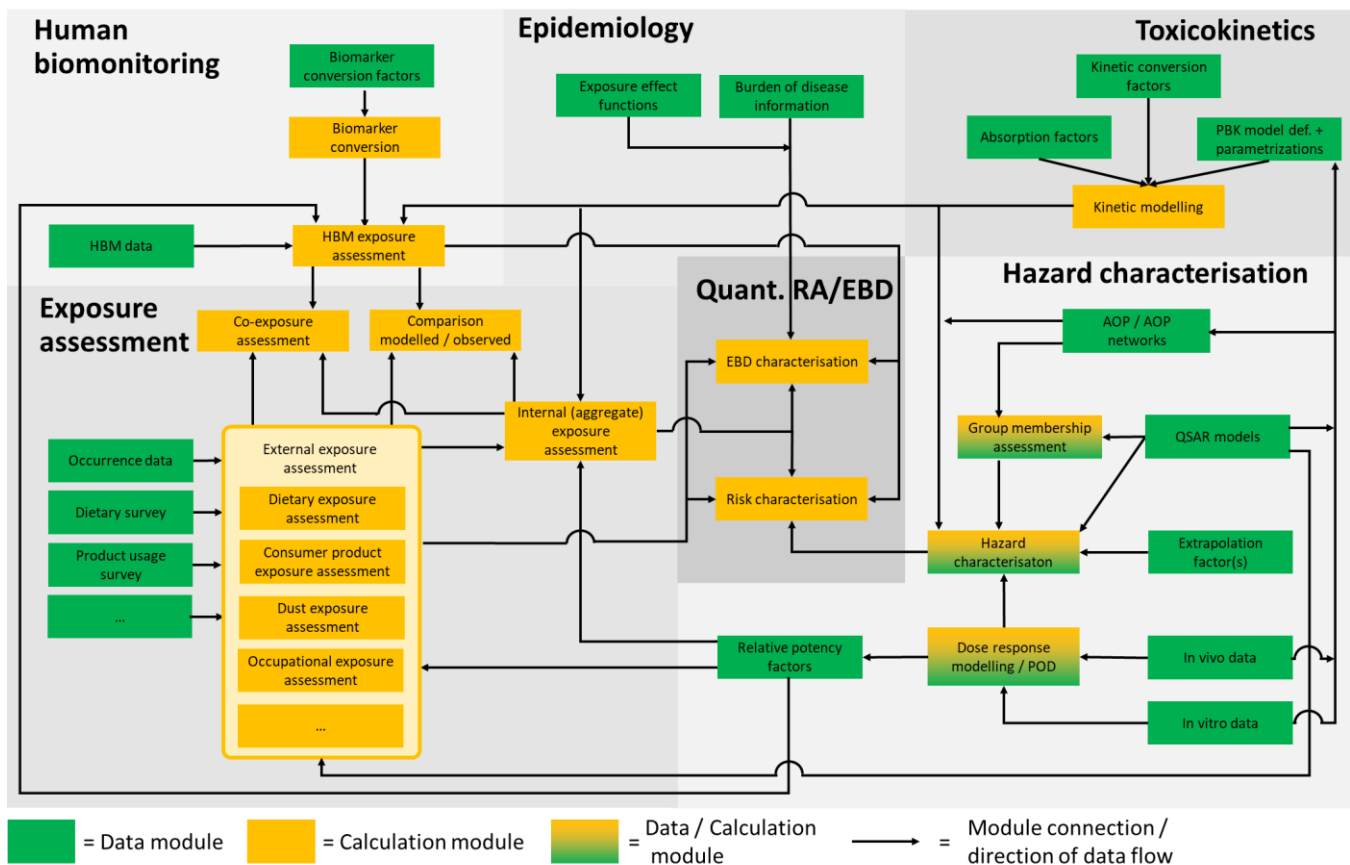


Figure 1 The conceptual framework of the PARC model network illustrates the (potential) computational steps used in integrative risk assessment workflows. It features a network of linked calculation models (yellow boxes, representing model classes and subclasses) and data models (green boxes), spanning various domains (grey areas).

In the conceptual framework, IRAs are broken down into a series of modelling activities. These activities, along with their input data, are represented as distinct modules – yellow boxes for modelling steps and green boxes for datasets – associated with specific domains, represented by grey areas. The arrows indicate the data flow where the output of one module is the input for another. For example:

- AEA is represented by linking data and model modules for multiple sources in the *external exposure assessment* module (e.g. dietary and consumer product exposure submodules). These outputs feed into the module *internal (aggregate) exposure assessment*, which aggregates the external exposure estimates at an internal level using the module *kinetic models*. The kinetic model could for example be a PBK model whose structure and parametrisation is provided to the module *kinetic models* as data from the module *PBK model + parametrisations*.
- The HBM exposure assessment module could form the basis for an assessment using measured exposures at an internal level. Its output could feed into the *risk assessment* module together with the output of *hazard characterisations* to assess to what degree the HBM concentrations exceed a human biomonitoring guidance value (HBMGV).
- The framework of quantitative in-vitro –to-in-vivo extrapolation is represented by module *dose-response modelling* with *in vitro* data as input. The dose-response (DR) relationship provides input to the module *hazard characterisations* together with the output of toxicokinetic (TK; i.e. modules *kinetic models* and *PBK model + parametrisations*) and *AOP / AOP networks* data to obtain in vivo hazard characterisations (HCs).

In the conceptual framework, uncertainty is considered a third dimension, with uncertainty propagating through the modules within a workflow (see section 4.2). The framework also supports tiered approaches. For example, it accommodates both low-tier and high-tier models for dietary exposure assessment, and includes kinetic modelling based on either low-tier kinetic conversion factors and high-tier PBK models. These modular, tiered components, enable the development of low-tier, high-tier, and hybrid workflows.

2.2 Modelling tools

The development of the PARC model network follows an iterative approach, where workflows are developed and linked based on (regulatory) needs from case studies within PARC. The workflows described in this deliverable integrate ten modelling tools (see sections 2.3 and 0). As illustrated in Figure 2a, these tools collectively cover all domains of the conceptual framework, which will be demonstrated through the workflows presented in section 0. Figure 2b highlights the different tools within the network. Some tools consist of a single model that maps to one domain, such as the EuroMix¹ and Westerhout PBK models [6, 7]. Other specialised tools, such as VEGA², function as toolboxes that remain within a single domain but include a large number of models (in this case, QSAR models). Finally, “broad” model platforms such as the Monte Carlo Risk Assessment (MCRA) toolbox³, INTEGRA and the source –to–outcome pathway (STOP) predictor⁴, incorporate multiple models spanning up to seven domains [8, 9]. These platforms currently serve as access points and user interfaces (UIs) for the workflows described in this Deliverable. STOP functions as the UI for environmental RA workflows, while MCRA and INTEGRA are both used for human RA workflows. While there is overlap, MCRA provides workflows for probabilistic aggregate RAs, whereas INTEGRA is currently focused on NAM-based RA. This distinction, however, is only a rough categorisation as the scope and applications of the platforms continue to evolve.

It is important to note that the tools presented in Figure 2b do not encompass all tools that are being considered in the broader network. Also, some existing tools are not listed because they are not yet actively used in the workflows described in section 0, despite having relevant connections within the network. e.g. the link between MCRA and PROAST for DR modelling in RA, which is briefly discussed in section 3.1.4. The list of integrated tools is expected to evolve over time as new functionalities are added, new tools become interoperable, and additional workflows are developed. For instance, multiple PBK models are being implemented using the PARC FAIR (Findable, Accessible, Interoperable, and Reusable) PBK standard (see section 4.1). Additionally, modelling tools that have already been linked may receive new functionalities to fill gaps in coverage. An example is the ongoing development of functionality for EBD assessments in MCRA (see section **Erreur ! Source du renvoi introuvable.**). Furthermore, future workflows may expand into new domains, such as environmental monitoring which was identified as relevant previously [1, 2].

To keep track of these developments and provide a clear overview of the modelling tools included in the model network, an inventory of these tools is maintained [1, 2]. In this inventory, structured data essential for building the model network is gathered for these tools, including general details, information on the models (e.g., the CRA domain), information on availability (including availability of source code) and licensing, and technical information (e.g., on versions, versioning, programming language(s)). We are currently exploring how this inventory can be made broadly accessible via the PARC FAIR data hub, PARCopedia⁵, and potentially through a user-friendly tool on the model network landing page⁶. If realised, such an interface would make the model network more tangible and enable users to efficiently search for relevant tools and workflows for specific problems – for example, listing all exposure assessment models, retrieving all FAIR PBK models for per- and polyfluorinated alkyl substances (PFAS), or identifying all workflows applicable to HBM data in the PARC HBM format. To support this, key characteristics of tools (and workflows) including general findability and accessibility, conceptual (such as the tool’s purpose) and technical aspects (e.g. supported data and communication standards) are being captured.

2.3 Workflows

As previously defined, a workflow in the modelling network consists of one or more computational (modelling) steps, executed by one or more modelling tools, to transform input into output data [2]. These outputs provide insights about the (health) impact of chemical exposure, typically in the form of figures and tables. Workflows are developed within a specific context. Multiple workflows may address the same aspects (purpose, covered domains, methodology applied) but differ in specific details.

¹ <https://github.com/rivm-syso/euromix-to-sbml>

² <https://www.vegahub.eu>

³ <https://mcra.rivm.nl/>

⁴ <https://www.niva.no/en/source-to-outcome-predictor>

⁵ <https://www.parcopedia.eu/tools/>

⁶ <https://www.parc-models.eu>

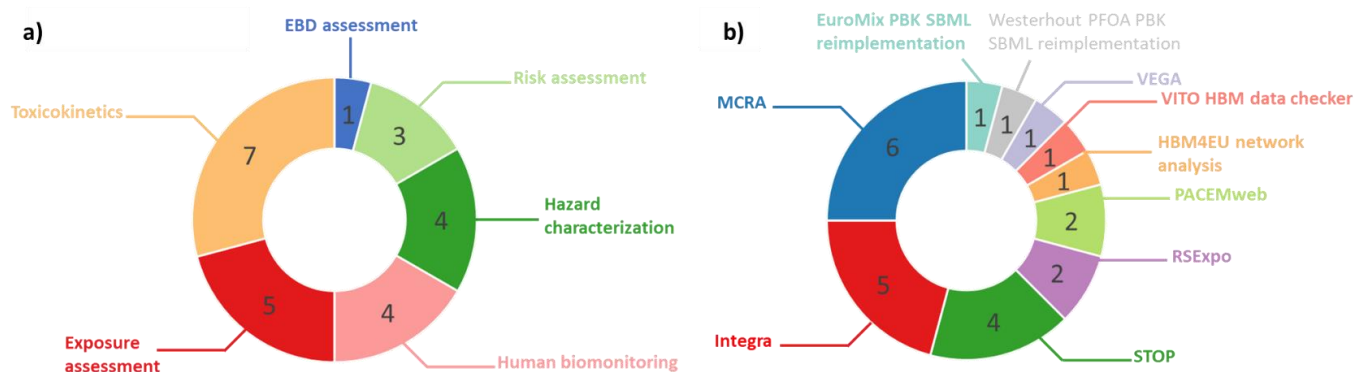


Figure 2 Overview of the modelling tools used in the workflows described in this deliverable, detailing (a) the number of tools within each domain of the conceptual framework, and (b) the number of domains associated with each tool. For clarity, environmental burden of disease (EBD) assessments and risk assessments are shown separately rather than a single domain as in Figure 1. The tools mentioned in this Figure are explained in more detail later in this document.

In this deliverable, we present in section 0 the workflows that were implemented or refined in year 3 of PARC. Table 1 presents an overview of these workflows including their purpose, links to other PARC tasks, and development stage. The workflows in this table are ordered based on their primary access point and their level of maturity within that access point. Some workflows are in *active use in a production environment* meaning that they are actively being applied by multiple stakeholders for assessments within and outside PARC. Others are in a *prototype* or *validation and refinement* phase and will require further development over time. While still in a development stage, workflows are currently versioned indirectly through the versioning of the tools and data formats they incorporate. As workflows progress towards release, we will place increased emphasis on explicitly versioning the workflows themselves, including tracking of the specific tool versions used within the workflow. This will become a focus in a later phase of the project.

Each workflow is developed within its own context, for its own specific purpose. However, the adoption of the conceptual framework facilitates their integration into broader assessments and thus supports the reuse of entire workflows or specific components within other workflows. For instance, the workflow for mixture risk assessment (MRA) based on HBM data (measured exposures) could be complemented by the workflow for AEA (modelled exposures) to help identify the sources and routes of exposure for key mixture risk drivers. This area will require further development and is discussed in section 4.

Table 1 Overview of the workflows developed or refined in year 3 of PARC. For completeness the table also includes the workflow for mixture selection at the bottom, which was developed in years 1 and 2, see [2].

Name (section)	Purpose	Primary access point	Modelling tools (currently)	PARC link	Development stage
HBM mixture risk assessment (3.1)	Human-health MRA based on HBM data.	MCRA	HBM data checker, MCRA, PARC FAIR PBK models	T6.2.3	Active use in production environment
Aggregate exposure assessment (3.2)	Human (internal) exposure assessment for multiple sources and routes	MCRA	RSExpo, PACEM, MCRA, PARC FAIR PBK models	T6.2.1, T7.3	In validation and refinement stage
Comparison of modelled exposures and HBM concentrations (3.3)	Comparison of modelled (dietary) exposures to measured (HBM) exposures	R (later MCRA)	R, MCRA, PARC FAIR PBK model	T6.2.1, T7.3	In validation and refinement stage
EBD assessment (3.4)	Human-health environmental burden of disease assessment based on HBM data	MCRA	HBM data checker, MCRA	T6.2.4	Early prototype
Chemical grouping (3.4)	Grouping of substances for screening / classification, prioritisation and risk assessment	MCRA	VEGA, MCRA	T8.3	Early prototype
Environmental risk assessment (3.6)	Link chemical releases from their sources to their potential biological effects.	STOP	STOP	T.5.1.2, T6.4, T7.2.2, T.7.3	Early prototype
NAMs for risk assessment (3.7)	Human health impact assessment assimilating NAM data	INTEGRA	INTEGRA	T8.3	Early prototype
HBM mixture selection (previous Deliverable)	Identification of real-life mixtures using HBM data	MCRA	HBM data checker, MCRA, RSExpo, network analysis R script	T6.2.3	In validation and refinement stage

3 Demonstration and validation of workflows

3.1 HBM mixture risk assessment

On a daily basis individuals are exposed to chemical mixtures through their diet and various other sources. While EFSA has made significant progress in addressing dietary mixture risks of plant protection products (PPPs), there remains a need for evaluations that cover all possible sources and routes of exposure. To address this, the PARC *real-life mixtures* project (T6.2.3) is exploring the application of HBM in MRA.

As described in AD8.4 [2], a workflow for HBM-based MRA was implemented in close collaboration with T6.2.3. The approach to mixture RA utilised in the workflow stems from this task (see [10]) and is based on the regulatory framework for retrospective dietary cumulative RA outlined by EFSA (2020). In addition, the workflow was aligned with the statistical principles for processing HBM data outlined in the statistical analysis plan of WP4. The workflow is **generic** with respect to the **scope of population, substances, health effect**, and considers different **exposure scenarios**, based on input data sources and settings.

This **workflow is actively used in a production environment in the context of T6.2.3 case studies** and was demonstrated to (regulatory) stakeholders including EFSA (e.g. alignment EFSA RACEMiC and ExpoAdvance roadmaps [11, 12]) and national experts. HBM study owners in the *real-life mixtures* project have been trained in the use of the workflow and have applied it to over 30 HBM data sets in case studies focusing on MRA of metals, pesticides and PFAS. Guidance for these applications was developed in T6.2.3 (see upcoming PARC deliverable D6.3).

This report provides an updated workflow description, with a particular emphasis on biomarker conversion, kinetic conversion and uncertainty analysis, building on the initial description of the workflow in AD8.4 [2]. Examples of biomarker conversion include calculating toxicologically relevant arsenic by summing up specific arsenic species or transforming total arsenic concentrations into measures of toxicologically-relevant arsenic. Kinetic conversions involve processes such as calculating lead levels in whole blood from urinary lead concentrations or determining external pesticide exposure from internal biomarker concentrations using reverse dosimetry.

3.1.1 Description

Figure 3 shows a refined version of the schematical depiction of this workflow presented in AD8.4 [2]. The basic description of the workflow remains consistent with the previous Deliverable. As in the previous Deliverable, **the MCRA web platform⁷ serves as the central UI** for applying the workflow. This platform was selected for its widely accepted interface for dietary MRA, supported by EFSA, and because other publicly available HBM modelling tools are limited. Rather than integrating with multiple external tools, the functionality of MCRA was expanded to fill in the gaps in modelling that were identified during the development of the methodology, building on foundations from the EuroMix project. For the privacy-sensitive HBM data it is important that the MCRA platform is secure, which is regularly evaluated in the context of EFSA's dietary RAs [13, 14]. In addition, a data controller – data processor agreement may be needed before a study owner can analyse their data on the platform to comply with GDPR regulations.

Briefly, **the workflow connects four modelling tools**, namely the VITO HBM data tools⁸, the MCRA platform, and two PARC FAIR PBK models. **The workflow integrates data and models across four key domains** illustrated in Figure 1: human biomonitoring, hazard characterisation, risk assessment, and toxicokinetics. This integration is implemented in a 2D Monte Carlo (MC) framework to (1) characterise variability in the calculation output (e.g. a risk distribution corresponding to variability between individuals) and (2) quantify the combined uncertainty about the output (e.g. 95% confidence interval for a percentile of the risk distribution), based on uncertainties in the inputs, which may include bootstrapping an input dataset and the use of probability distributions for uncertain inputs [8, 10]. The red text in Figure 3 indicates in which step of the workflow uncertainties are integrated. Note that this framework is also applied in MCRA for dietary MRA of pesticides, following the methodology of EFSA [15, 16]. The primary input for the workflow is an HBM dataset which is first converted from a study-specific format to the PARC harmonised format for HBM data using VITO tools.

⁷ <https://mcra.rivm.nl>

⁸ <https://hbm.vito.be/human-biomonitoring-data-services>

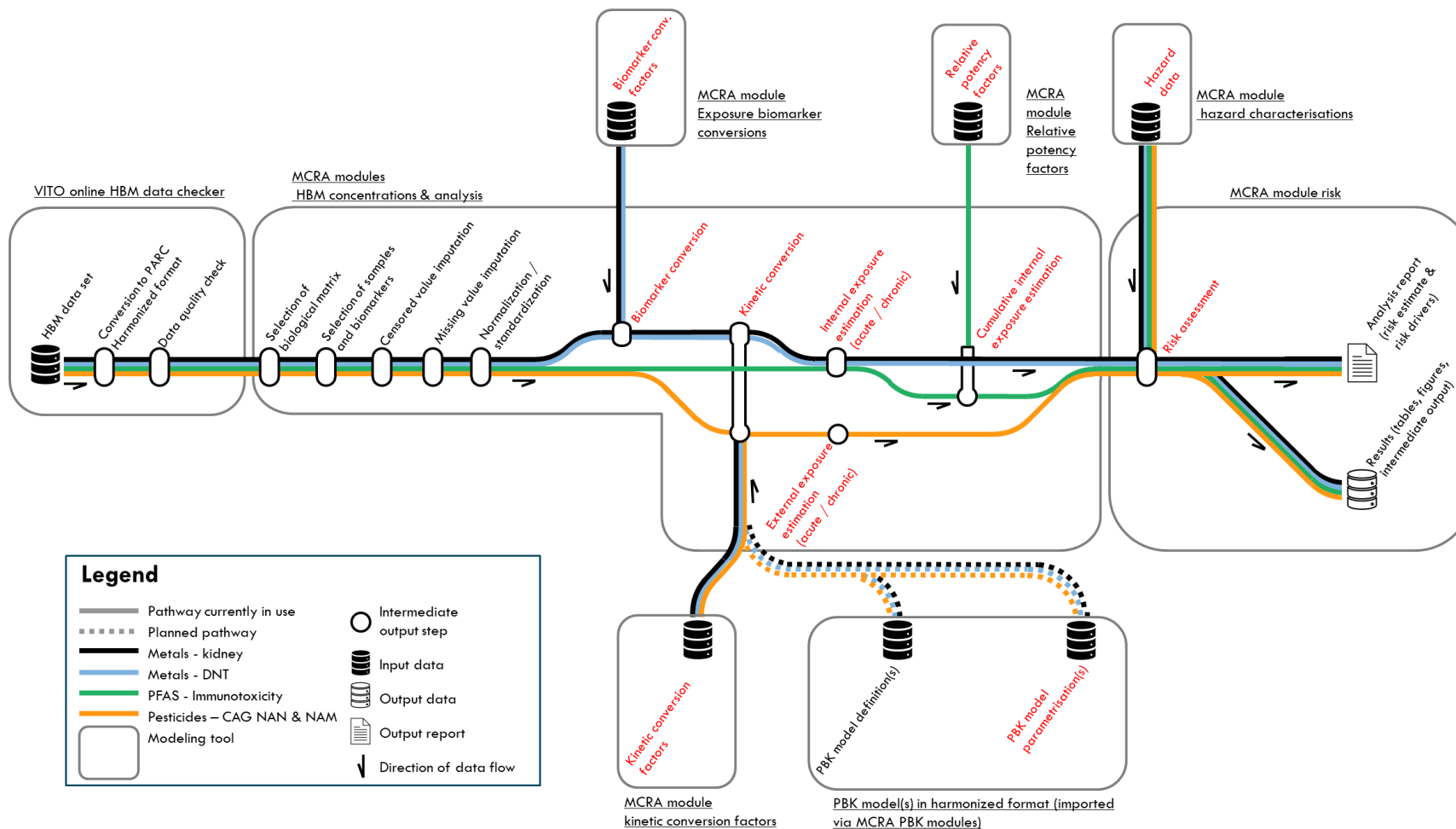


Figure 3 Schematic display detailing the workflow for HBM (mixture) risk assessment, emphasising the data, modelling steps and software tools used. Four examples of distinct pathways through the workflow are presented, with the chosen pathway depending on the data and input settings. Uncertainties are quantified and propagated at each intermediate output step highlighted in red.

The data are manually uploaded to the MCRA webplatform. Next, the data undergo filtering and pre-processing in the MCRA modules *HBM concentrations* and *HBM analysis*, following the WP4 statistical analysis plan. Some details were outlined in AD8.4 [2]. Here we focus on biomarker and kinetic conversions.

Biomarker conversions are used to transform the measured biomarker into the one relevant for RA. These conversions are incorporated into the workflow using a dedicated data format that supports any conversion expressed as a linear combination of the measured biomarkers. Different conversion factors can be specified for distinct population sub-groups (e.g. based on age and sex) and can optionally include a probability distribution to account for variability in the conversion factor within each sub-group. Examples include summing up arsenic species As(III), As(V), MMA and DMA to toxicologically relevant arsenic, or converting total arsenic to toxicologically-relevant arsenic using a beta distribution representing variability in the population. This distribution was obtained by application of beta regression of total arsenic values on toxicologically-relevant arsenic values that were observed in several HBM studies in which both were measured (see upcoming PARC Deliverable D6.3 for more details).

Kinetic conversions apply a TK model to the measured biomarkers to estimate the concentration of a biomarker in a different biological matrix or to estimate the external exposure to its parent compound. The workflow supports low-tier conversions using conversion factors, with plans to incorporate higher-tier conversions using PBK models following the PARC FAIR PBK standard (see sections 3.1.4 and 4.1). Similar to the biomarker conversions, kinetic conversions are specified by the user in a dedicated data format as any linear combination of the measured biomarkers. In a T6.2.3 case study on pesticides, reverse dosimetry is performed in this way, with conversion factors specified at the subgroup level, including uncertainty.

The final step in the *HBM analysis* module involves estimating acute or chronic exposures, and, optionally, cumulative exposures using (internal) relative potency factors (RPFs), see AD8.4 [2]. These estimates are then transferred to the MCRA *Risks* module, which estimates the variability distribution of risk across the population and identifies the primary substances contributing to the risk. The RPFs and HCs are provided to the system in a dedicated data format. Uncertainties can be specified for the RPFs. Different HCs, including associated uncertainties, can be specified for population groups. For example, in the case study on PFAS, age-dependent HBMGVs were used in this manner. Note that the *Risks* module supports different mixture risk metrics ranging from lower-tier sums of hazard quotients (HQs; e.g. modified reference point index, mRPI) to higher-tier cumulative risks based on RPFs, see below.

Additional details about these steps and the MCRA modules involved are provided in the MCRA documentation⁹ and the scientific publication of the new MCRA functionality for HBM-based MRA that is in preparation.

3.1.2 Demonstration

A first demonstration of the workflow was provided in AD8.4 for the metals case study focusing on kidney effects (black line in Figure 3). This demonstration was based on artificial data (based on characteristics of a real HBM data set) since the results of the case study had not yet been published at that time. Therefore, the demonstration does not represent a real RA. A similar approach is followed here, focusing on the application of the workflow for the PFAS case study on adverse effects on the immune system. Results of the application of the workflow to real data are presented in the upcoming PARC Deliverable D6.3 and the corresponding publications that are in preparation.

Figure 4 presents the MCRA UI for setting up the workflow. As described in AD8.4, a menu for selecting one of the relevant MCRA modules is visible in Figure 4a, while its corresponding data inputs and/or settings are shown in the main window. In this case, the *Risks* module is presented where the user specifies the exposure type (in this case chronic) and the risk metric. The risk metric is defined by the options *Risk characterisation ratio* and *Cumulation setting*. In this case, a cumulative exposure is estimated using cumulation setting *RPF weighted*. The risk metric is obtained by dividing the cumulative exposure by its corresponding HC (risk characterisation ratio is set to *exposure/hazard*). Note that this general mechanism also allows for specification of several risk metrics such as HQs and hazard index (HI), the margin of exposure total (MOET), the reference point index (RPI), and the mRPI.

⁹ <https://mcra.rivm.nl/documentation/>

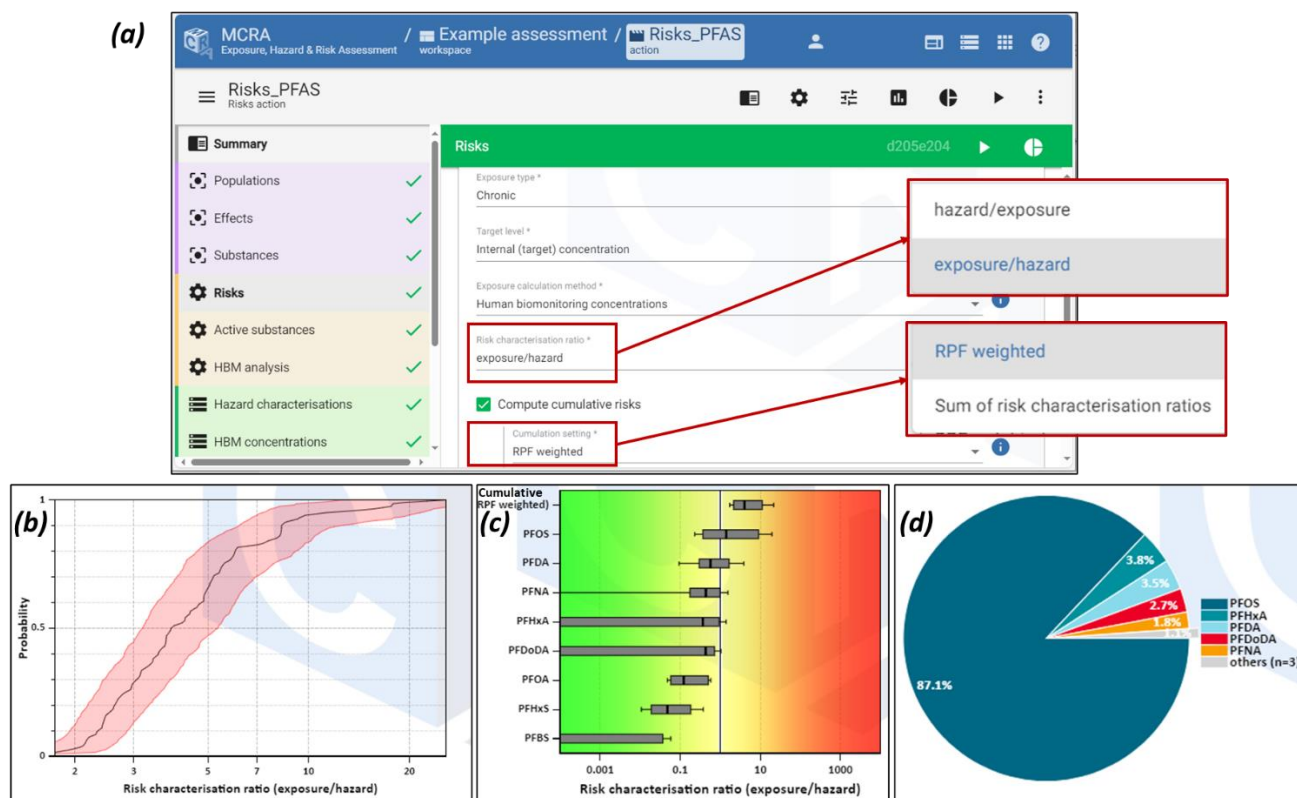


Figure 4 Example of the (a) the user interface for the MRA workflow, displaying options for specification of the risk metric; (b) a plot of the estimated cumulative risk distribution, showing the variability between the 5th and 95th percentiles as a grey bar, and the 95% uncertainty bounds as whiskers, corresponding to the lower 2.5% bound of the p5 and the upper 97.5% bound of the p95, respectively; (c) box plots of the cumulative risk distribution with those of the individual substances involved in the assessment; and (d) a pie chart highlighting the contribution of each substance to the upper percentile of the risk distribution.

Figure 4b-d illustrates examples of the graphical output generated by this workflow. Panels b and c display different visualisations of the estimated risk distribution including uncertainty bounds. In this example (based on artificial data), the entire population appears to be at risk from cumulative PFAS exposure. Panels c and d allow users to examine the contribution of each substance to the overall risk which is a first step towards exploring risk mitigation (see section 3.1.4). In addition to these graphical outputs, MCRA provides tables with more detailed data, such as estimated risk at various percentiles within the distribution. Intermediate results are also accessible. An analysis report summarising both, main and intermediate, outputs can be exported in PDF or HTML format. See AD8.4 for more details about the outputs of the workflow [2]. Furthermore, the workflow setup, including data files if desired, can be exported (and uploaded) for reproducible reanalysis at any time.

3.1.3 Validation and acceptance

As described in AD8.4, automated unit tests and regression tests were implemented to ensure the consistency of the workflow outputs across new versions of the MCRA software [2]. These tests cover a board range of scenarios, incorporating various data sets, models and settings, and are partially based on the case studies from the T6.2.3 *real-life mixtures* project.

In addition to this internal validation, the previous Deliverable focused on external validation of the workflow. For this purpose, the outcome of the workflow was compared against an independent implementation in R. This R implementation did not implement the general workflow, but rather the specific pathways through the workflow for the T6.2.3 case studies.

In the past year the workflow has been applied to numerous HBM data sets by study owners in T6.2.3 in different case studies. As a result, the focus of validation has shifted from external validation to acceptance of the workflow.

Generally, the workflow was directly applicable to all data sets, though minor changes were sometimes needed (e.g. when HBM studies contained follow-up data several years later). As part of the acceptance testing, the workflow has been and will continue to be presented to other stakeholders, such as EFSA.

3.1.4 Next steps

HBM-based MRA accounts for all sources and routes of exposure. However, to explore risk mitigation options in cases of significant risk, it is necessary to identify the sources and exposure routes of the mixture risk drivers. This requires reverse dosimetry in combination with external (aggregate) exposure modelling. Consequently, efforts will concentrate on linking this workflow with the AEA workflow presented in section 3.2. This will also integrate the T8.3 effort for FAIR implementation of PBK models (section 4.1). Developing these links will contribute to the emergence of the PARC model network as a digital ecosystem that connects models and data. Additional links to other workflows are anticipated, including the co-exposure assessment workflow for identification of real-life mixtures to base assessments on (see AD8.4 [2]) and the EBD assessment workflow (section 3.3).

Through the collaboration with PARC T6.2, efforts will also focus on disseminating the workflow to (regulatory) stakeholders. This will involve demonstration of the workflow and presenting the results, e.g. through a dedicated dashboard.

As noted in section 3.1.2, the workflow links to HCs and RPFs through data import functionality, thereby indirectly linking to DR models. Future developments, as outlined in AD8.4 [2], may include deriving HCs or RPFs directly within the workflow from *in vivo* or *in vitro* DR data. Tools like the PROAST package, which is already linked to MCRA, could be employed for this purpose.

3.2 Aggregate exposure assessment

Individuals are exposed to chemicals in both general and occupational environments, and these exposures occur through various routes (oral, dermal, inhalation) and originate from diverse sources, such as the diet, the use of personal care products (PCPs), air, soil, dust, etc. There is great interest in assessing the total exposure in a population factoring in all relevant sources and routes. PARC task T6.2.1 is developing an integrative strategy for such AEA. This is in line with recent initiatives from EU agencies such as the EFSA ExpoAdvance roadmap [12]. Discussions on alignment of efforts between T6.2.1 and EFSA ExpoAdvance are ongoing.

As described in AD8.4, a workflow for probabilistic aggregated exposure assessment is being developed in the PARC model network [2]. The aim is to develop a workflow that is generic with respect to the scope of the population, the substances and the sources and routes considered. It should be applicable for occupational and general-life exposure scenarios. The workflow will be applied in T6.2.1 case studies with aggregate exposure to PFOA and cadmium in the general population first in view.

This report provides an updated workflow description, with a particular emphasis on implementation of external exposure assessment models from the RSEXPo tool [17, 18], and the options for conversion of external exposure estimates to the internal level. Examples include the use of absorption factors to estimate aggregate exposure at a systemic level, or kinetic models to estimate aggregate exposure at an internal concentration (e.g. in blood or urine). The options for kinetic models range from low-tier kinetic conversion factors (see also section 3.1) to higher-tier pluggable PBK models via the PARC FAIR PBK standard (see section 4.1).

3.2.1 Description

The workflow integrates data and models across two key domains (as illustrated in Figure 1): **exposure assessment and kinetic modelling**. Figure 5 provides an updated schematical depiction of this workflow, which builds upon the version presented in AD8.4 [2]. The fundamental structure of the workflow remains consistent with the previous Deliverable. As before, the MCRA web platform serves as the central UI for applying the workflow.

As shown in Figure 5, the workflow starts with external exposure modelling from various sources and routes. Currently, it incorporates three exposure estimation tools, namely MCRA, PACEM [19], and RSEXPo. Together, these tools cover all exposure routes and the following sources: diet, consumer products, dust, soil, air, and pet fur. The current version of the workflow supports exposure assessment for diet, consumer product and dust, while inclusion

of the other sources covered by the tools is planned for the next update. Future updates might also integrate additional exposure assessment tools covering other sources and occupational scenarios.

Exposures originating from consumer products are assessed using PACEMweb¹⁰. The exposure estimates are manually exported into a harmonised data format and subsequently transferred to the MCRA platform using the module *Non-dietary exposures*. See AD8.4 for more details. Dietary exposure estimates are obtained through the MCRA module *Dietary exposures* and several sub-modules thereof. More information is also provided in AD8.4 and the MCRA documentation¹¹.

Dust exposure assessments are conducted using the new MCRA module *Dust exposures* and several sub-modules thereof. This module contains a reimplementation of the RSEXpo dust model. Due to RSEXpo's design as an RShiny program with a French-language UI, (automatic) linking between MCRA and RSEXpo was deemed impractical. As a result, RSEXpo models are being reimplemented in MCRA in close collaboration with its developers. The *Dust exposures* module estimates chronic exposure to dust based on input data such as dust concentration levels and dust exposure determinants. The exposure determinants can be specified as a function of age and sex. Detailed information about the calculations and equations used can be found in the MCRA documentation¹²,

External exposure estimates are provided as input to the MCRA module (*Internal*) *exposures*, where they are aggregated at a specified internal level (i.e. systemic exposure or internal concentrations) in two steps. Typically, both dietary and non-dietary exposure estimates are based on data from independent surveys (e.g. dietary consumption surveys and surveys on PCP usage). As a first step, the populations/individuals considered in the surveys are matched using one of three methods: random linking of individuals, random linking of individuals within subgroups (specified by properties such as age and sex), or linking individuals based on unique individual identifiers. After matching, the external exposure estimates are combined for each linked individual. In addition, the units in which each exposure estimate was obtained are aligned such that they can be easily aggregated at the internal level. Systemic exposures are calculated using absorption factors, while kinetic conversion factors are applied to aggregate exposures at a specific target level such as urine or blood.

For high-tier conversions, the workflow incorporates functionality to make use of PARC FAIR PBK models. These models can be uploaded by the user into the system and directly used in the workflow. This has been demonstrated for the generic EuroMix PBK model¹³ (already available in MCRA and TKplate [20]) and a simple PFAS PBK model implementation based on the PFOA model by Westerhout et. al [6, 7]. Both models were converted to the systems biology markup language (SBML) and annotated following the currently being developed FAIR PBK harmonised modelling standard (see section 4.1). Similarly to the workflow in section 3.1, the integration of these models is being implemented in a 2D MC framework to estimate variability in the exposure in the population of interest and assess the uncertainty in these estimates.

We refer the reader to the MCRA documentation for more information regarding the internal exposure estimation and the use of PBK models¹⁴.

3.2.2 Demonstration

The initial application of the workflow was demonstrated in AD8.4. Here, we extend that example by focusing on chronic AEA for PFOA including dust as an additional exposure source in addition to diet and PCP. Please note that this is a technical example demonstrating that the workflow integrates various exposure assessment models and PBK models. This example is not a finalised assessment, and validation is required for both, the data and model configurations.

¹⁰ <https://www.pacemweb.nl/>

¹¹ <https://mcra.rivm.nl/documentation/10.1.3/modules/exposure-modules/dietary-exposures/index.html>

¹² <https://mcra.rivm.nl/documentation/10.1.3/modules/exposure-modules/dust-exposures/index.html>

¹³ <https://github.com/rivm-syso/euromix-to-sbml>

¹⁴ <https://mcra.rivm.nl/documentation/10.1.3/modules/exposure-modules/internal-exposures/internal-exposures-calculation.html#internal-exposures-calculation>

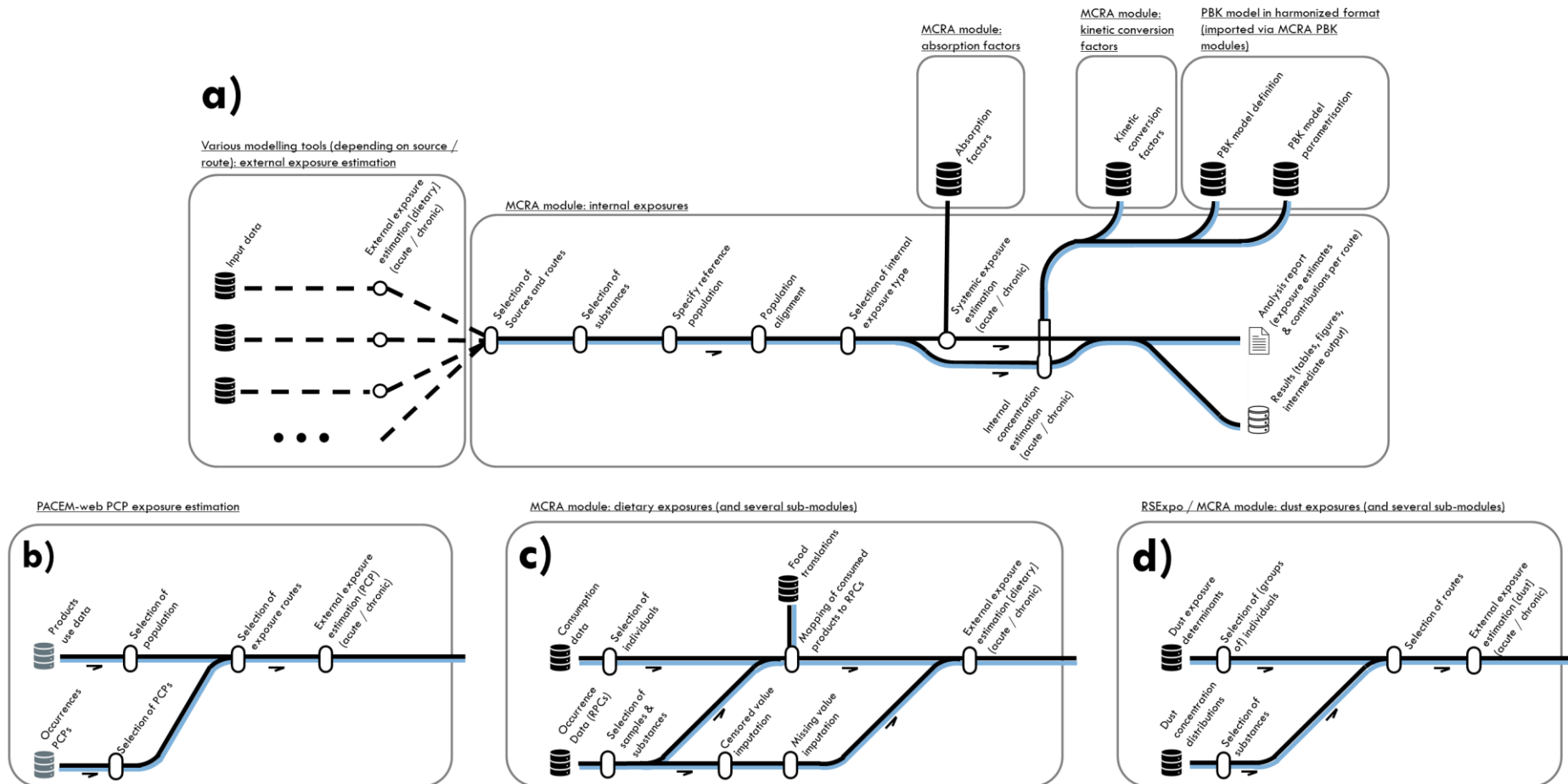
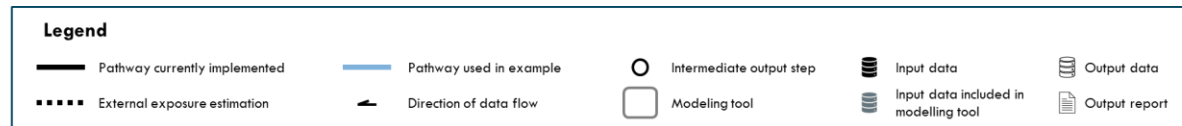


Figure 5 Schematic display detailing the workflow for aggregate exposure assessment, emphasising the data, modelling steps and software tools used. The main workflow is displayed in panel a) with external exposure estimates derived from different modelling tools based on the sources and routes of interest. Panels b) to d) present three examples of external exposure estimation tools that are connected to the main workflow, with additional tools to be linked in the future. The specific pathway through the workflow used in this Deliverable is highlighted in blue.

The demonstration aggregates exposure to PFOA via PCP, food and drinking water and dust in the general population. Dietary exposures were estimated in MCRA following the approach of Schepens et al. [21]. Dermal exposures from use of PCPs were assessed using PACEMweb, which incorporates survey data on PCP usage patterns [19]. Dust exposures were estimated using the new MCRA module for dust exposures using preliminary concentration values and exposure determinants from T6.2.1.

The exposure estimates were aggregated in the MCRA internal exposures module using a demonstrative implementation of a FAIR PBK implementation in SBML, based on the model of [7]. For each (simulated) individual, internal steady-state concentration levels in venous blood plasma were obtained by running simulations using the external daily exposure estimates for the different routes. For this demonstration, a limited number of only 1000 days were used for simulation. For realistic PFAS assessments, longer simulations should be considered to reach steady-state concentration levels. Figure 6 illustrates the MCRA UI that is used for AEA, with the option to include routes and sources of exposure of interest. The figure also shows a histogram of the internal exposure distribution (with estimated contributions of the different routes), a pie chart showing the contributions of the different routes to the total distribution, a scatter chart showing the relationship between (total) external (systemic) exposure and the internal steady-state concentration levels for all simulated individuals, and the internal concentration time-series of one of the simulated individuals, here showing that a steady-state concentration level was not yet reached after 1000 days.

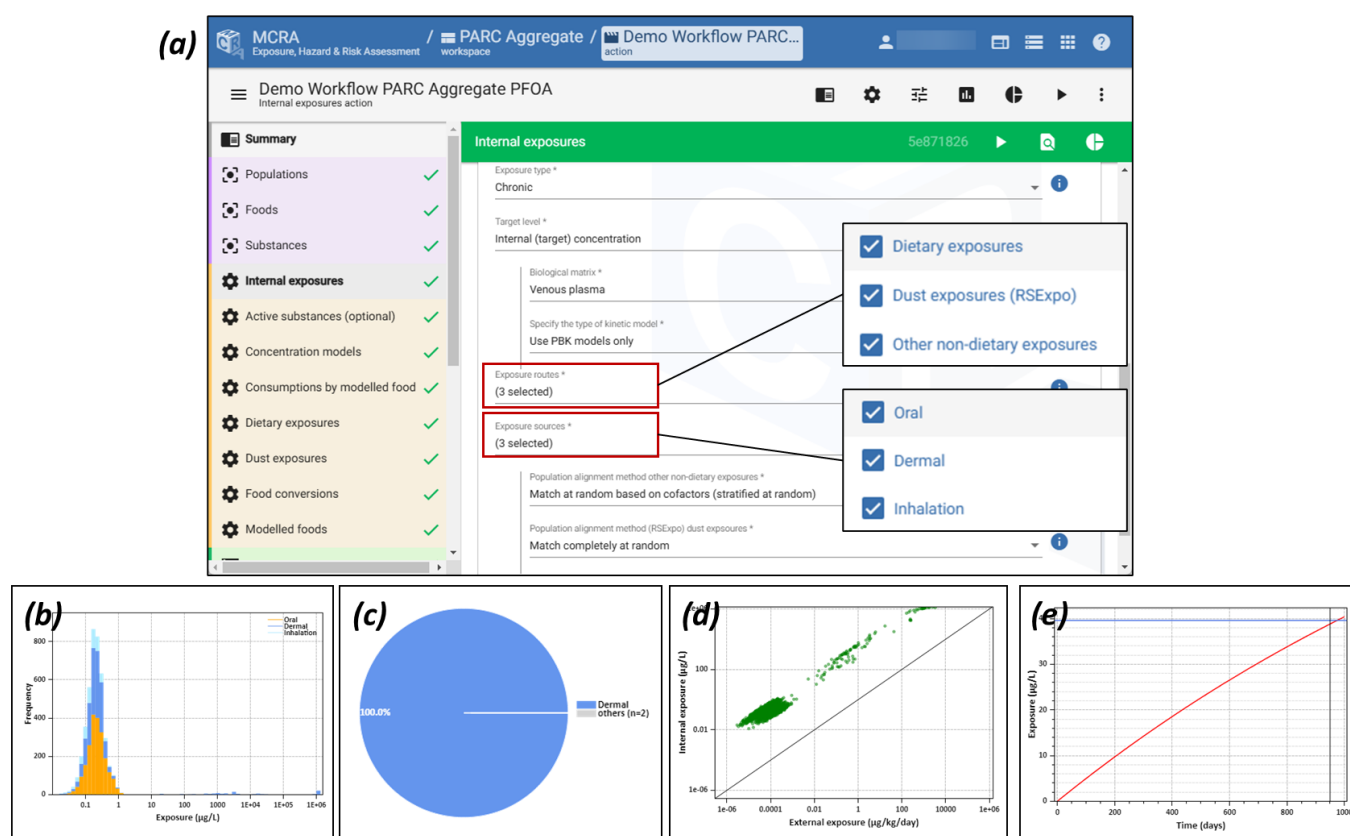


Figure 6 Example of (a) the user interface of the workflow for aggregate and internal exposure assessment, displaying the options for specifying the routes and sources of exposure and the internal target surface of interest; (b) a histogram of the internal exposure distribution and contributions of the different routes; (c) a pie chart of the contributions of the different routes to the total distribution; and (d) a scatter plot showing the relationship between the total (systemic) external exposure and the internal (steady-state) concentration at the target (venous blood plasma) after for all simulated individuals after 1000 days of exposure; (e) internal concentration time-series of one of the simulated individuals, here showing that a steady-state concentration level was not yet reached after 1000 days.

3.2.3 Validation

Validation of the workflow is currently underway. Note that this does not include individual modelling tools that are used outside the workflow (PACEMweb or specific PBK models). The MCRA dietary exposure estimation module has already been validated in the context of the EFSA methodology for dietary exposure estimation to mixtures of pesticides (refer to EFSA papers for dietary). The newly developed MCRA module *Dust exposures* has been validated by comparing its output to the RShiny program RSEXPO, which contains the same model. Additionally, internal unit and regression tests for the entire workflow are in development. The application of the workflow will be further validated within T6.2.1 case studies.

3.2.4 Next steps

In year 4 of PARC, the workflow will be expanded to include more exposure sources. Initially, exposure via air and soil will be included using the models from the RSEXPO tool. The new MCRA dust module will serve as the blueprint for implementing these sources. In parallel, we will begin integrating support for occupational exposure scenarios following the same approach. Subsequently, the integration and linking of other models and modelling tools will be explored.

Another focus will be a refinement of the integration of FAIR PBK models in the workflow (see section 4.1).

Over time there is the intention to link the (*Internal*) *exposures* module to the *HBM analysis module* described in section 3.1. As shown conceptually in Figure 1, this would allow for making comparisons between modelled and measured exposures. Such comparisons would 1) validate the aggregate exposure estimates at the internal level by assessing whether they align with the values that are observed in relevant HBM data, and 2) facilitate the analysis of sources and routes for key mixture risk drivers within an HBM-based mixture RA.

3.3 Comparison of modelled exposures and HBM concentrations

A theoretical framework for quantification, assessment, propagation, and comparison of uncertainty within the integrative models network is being developed in T8.3 and demonstrated through the case study of T7.3.2, "Uncertainty in PFAS Exposure." In Y2, a workflow for application of the framework within the context of the T7.3.2 case study was presented [2]. The workflow aims to compare modelled exposures to measured exposures, in particular daily intakes derived from reverse dosimetry based on HBM data to estimates following from a dietary exposure assessment model. In Y3, we compiled an internal overview of methods for uncertainty assessment in PBK models, with a particular focus on the propagation of variability and uncertainty in both PBK and exposure models, as outlined in the case study, and ran first rough simulations on our cohort dataset. This section presents an update on the workflow for running the first simulations. A general discussion on uncertainty analyses in the model network is presented in section 4.2.

3.3.1 Description

A detailed description of the workflow is presented in AD8.4 [2]. Briefly, the workflow combines elements of the workflows for HBM-based MRA (section 3.1) and AEA (section 3.2). It is presented in Figure 7 and connects models from two domains of the conceptual model (Figure 1), namely human biomonitoring and exposure assessment.

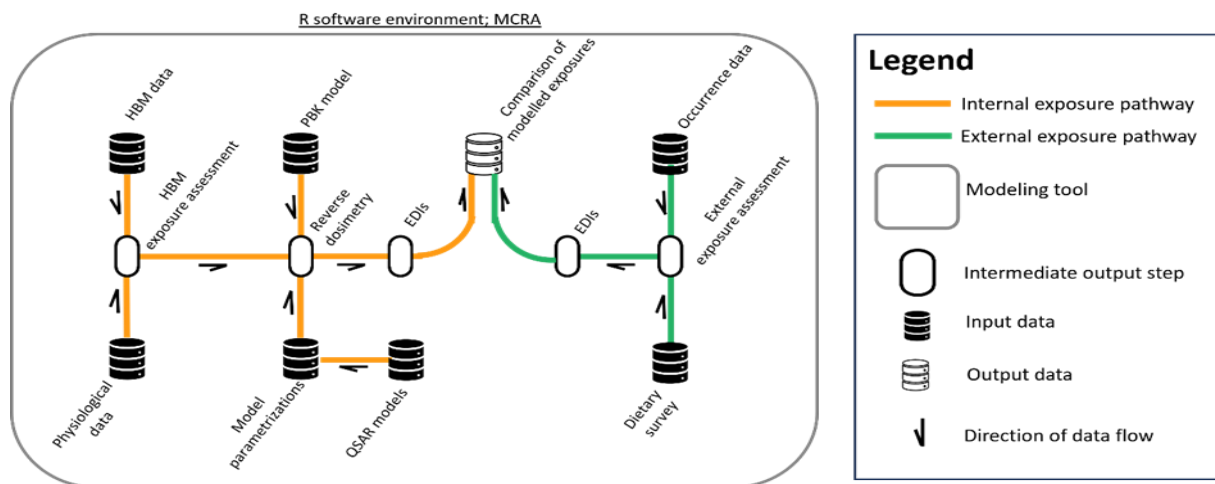


Figure 7 Schematic overview of the case study “Assessment of uncertainty in PBK models of PFAS exposure in humans and reverse dosimetry (uncertainty in PFAS exposure)”.

The workflow consists of two main data flows: HBM exposure (internal pathway) and external exposure (external pathway). The HBM exposure pathway aims to provide individual estimated daily intakes (EDIs) (e.g. for PFAS) using reverse dosimetry by a PBK model including quantification of their uncertainty as well as calibration of the model parameters. On the other side, the external pathway aims to estimate individual EDIs of the same population and substance (e.g. PFAS) using appropriate occurrence data and dietary patterns with a thorough quantification of their uncertainty. The final step of the workflow is the comparison of the corresponding EDIs and their uncertainties. The uncertainty assessment is conducted by several different methods throughout the entire data flow (input data, calculator modules, etc.) and represents the main outcome reflecting the focus of the case study. The workflow is currently implemented in R¹⁵. In the final stage, the workflow will be integrated within the framework and workflows implemented in MCRA.

As seen from Figure 7 and Figure 5 (Section 3.2), the workflow of the case-study “Uncertainty in PFAS exposure” is integrated into the AEA study, as several modules overlap between the case studies. Both workflows include two main pathways: external and internal exposure estimation. The current study includes only dietary external exposure while the AEA study includes additional exposure sources, such as inhalation and dermal intake. The primary difference lies in the direction of the data flow: in the AEA study external exposure estimates serve as inputs for internal level estimations using PBK models and absorption/kinetic factors (forward dosimetry). This approach also allows for comparing internal exposure estimates with measured internal concentrations (“ground truth”) when available. However, reversing the workflow direction - using internal exposure estimates for external exposure estimates (reverse dosimetry) – overlaps with the case study where internal blood levels serve as input for deriving EDIs estimates that are compared with modelled external exposure EDIs estimates. Thus, despite sharing similar modules, the studies apply different data flows. In this sense, the present workflow overlaps more with the kinetic conversion principle for HBM data outlined in section 3.1. The AEA case study is broader, including multiple exposure sources and modelling tools for internal concentration estimation. In other words, the case study represents a subset of these workflows, primarily focusing on uncertainties in input data, model parameterisation, and uncertainty propagation, serving as a practical case study for this purpose. The uncertainty assessment methodology will be extended to various PBK models and compounds. On the other hand, the AEA study aims to develop an accurate tool for modelling internal concentration estimates based on external exposure data. Relevant outcomes of the current study will be implemented in MCRA and will complement the results of other workflows by providing information about the accuracy of the outcomes.

There are several steps in the uncertainty assessment for internal EDIs in the “Uncertainty in PFAS exposure” case study. Firstly, global sensitivity analysis of the PBK model will be applied in the first step. The primary goal is to identify the most influential input parameters of the model. For this step, the method of Extended Fourier Amplitude Sensitivity Test (eFAST) [25] was selected; however, other methods such as Morris [26] or MeFAST [27] should be tested, too (see section 4.2). The eFAST method uses Fourier decomposition and integer coefficient

¹⁵ https://github.com/DariaSapunova/MU_PFAS_uncertainty_case_study

approximations for the variance function. It is relatively computationally efficient compared to other global sensitivity analysis methods. Secondly, model calibration will be applied, based on the results of the global sensitivity analysis. The calibration will be conducted to determine optimal combinations of input parameter values. For this step, the Bayesian Metropolis-Hastings (MH-MCMC) algorithm [28] was tested in Y3. This algorithm generates a random walk from any parameter distribution and evaluates the distance between predicted and observed outcomes. MH-MCMC will thus estimate the most probable values for the input parameters over the assessed population. Although simple and widely used, MH-MCMC requires careful optimisation of key elements, such as the acceptance rule, step sizes, and the initial starting point, to improve efficiency. Additionally, it seems to be computationally demanding.

The uncertainty assessment of external exposure was performed in Y3, using both the MC simulation method [29] (numerical) and an approximative analytical method based on the Fenton-Wilkinson approximation [30] for summing log-normally distributed concentrations of PFASs in food. The MC method is computationally demanding, but more robust compared to the analytical approach which suffers mainly due to zero-inflated consumption frequency data. At this point in time, we are implementing the Tweedie distribution to cover the probabilistic nature of the consumption frequencies data. Both analytical approximation and MC methods provide a distribution of the estimated EDI values for each individual.

The methods that are being developed for uncertainty assessment will help make exposure estimates more reliable and improve understanding of how model parameters vary. These improvements will be useful for applying it to different PBK models and compounds in the future. In Y3, the focus was on external exposure uncertainty and model calibration using MH-MCMC. External exposure uncertainty was assessed through MC simulations and analytical method based on the Fenton-Wilkinson approximation.

3.3.2 Demonstration

As described in D8.4, the workflow of the “Uncertainty in PFAS exposure” case study is a part of the T6.2 framework. The case study aims to perform qualitative and quantitative estimation of uncertainties using the case study as a practical case for testing the methodology. The relevant outcomes will be implemented in the PARC integrative model toolbox in collaboration with T8.3. In this application, the workflow aims to compare estimates of PFAS EDIs from internal blood concentrations of the European population and EDIs from external dietary exposure along with an uncertainty assessment of the external part. Thus, in Y3 an uncertainty assessment was primarily done for the external exposure part of the workflow.

The external exposure assessment model was developed for estimation of PFAS EDIs from dietary patterns and occurrence data. The MC simulation method (numerical) or analytical method paired with the Fenton-Wilkinson approximation are used to estimate the overall exposure and its uncertainty. The external exposure model was performed on Czech adults and will be expanded to the other European countries.

To estimate PFAS EDIs from blood levels of the same individuals, a modified PBK model based on the PFOA and PFOS model developed by Loccisano et al. was utilised [31]. However, this model does not reflect the state of the art and was chosen for an illustrative demonstration of the workflow application. In collaboration with T6.2.2, work on adapting a model developed by Husøy et al. for all life stages with a comprehensive set of physiological and chemical parameters [32] is ongoing. The parameters’ real distributions were collected during Y3 and their distributions will be finalised in Y4. However, for the illustrative demonstration a generic set of parameters by Loccisano was used. These limitations stated above should be accounted for when interpreting the outcome of the demonstration.

In meantime, a one-dimensional MH-MCMC algorithm was applied to calibrate PFAS EDIs to match observed blood concentrations. MH-MCMC is a type of Markov Chain Monte Carlo (MCMC) method, it proposes possible points in the parameter range and decides if to accept them based on probability derived from likelihood and prior information. This algorithm is applied here as a calibration of one parameter only (EDIs in our case); however, in the final implementation it will explore different combinations of values of the input parameters that will match the output (blood concentrations).

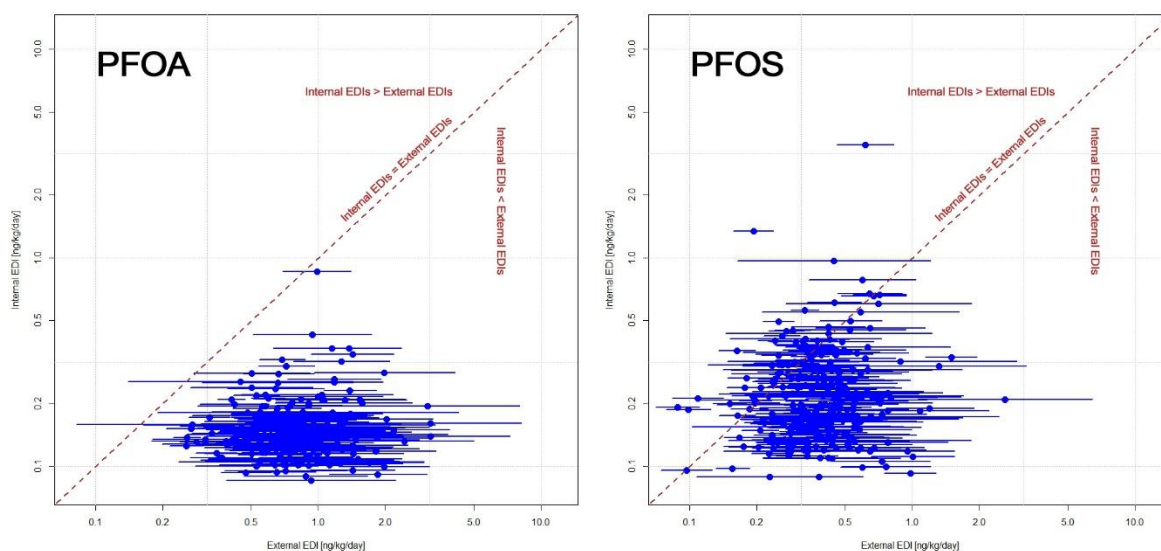


Figure 8 (a) Illustrative comparison of PFOA and PFOS EDIs (internal vs external*) by Czech population individual. The blue solid lines represent error bars (± 1 GSD from the GM) for EDIs derived from external exposure. Internal EDIs = preliminary EDIs estimates from blood concentration that were derived using a PBK model and the Bayesian Metropolis-Hastings algorithm (parametrisation in collaboration with T6.2). External EDIs – preliminary EDIs estimates that were derived using external exposure assessment model with the analytical method (summing log-normal distributions using Fenton-Wilkinson approximation) to assess EDIs uncertainty.

Figure 8 demonstrates an illustrative comparison of EDIs for PFOA and PFOS generated from external dietary exposure assessment method (analytical) and EDIs generated from the PBK model with a generic set of parameters using MH-MCMC algorithm for EDIs calibration for the Czech adult population. Since only dietary exposure was estimated, neglecting other possible exposure sources, EDIs estimated from blood levels are expected to be higher than EDIs estimates from the dietary exposure. However, in this preliminary comparison, most of the participants have EDIs from dietary exposure higher than EDIs derived from the blood levels – this will be discussed during further investigation in Y4. Error bars in the plots (± 1 GSD from the GM) (Figure 8) demonstrate external EDIs uncertainty assessed based on the analytical method. The uncertainty of the PBK reverse dosimetry was not assessed yet since the distributions of PBK model parameters were not finalised in Y3. As seen from the plots, uncertainty is not consistent across the individuals for both PFOA and PFOS.

The overall inconsistency stated above could be explained by the limitations of this preliminary application:

- the PBK model does not fully represent the current state of the art (development of the model will continue in Y4, including at least uncertainty estimates and menstrual blood loss for women);
- PFAS occurrence data is primarily available for Western Europe, as there is a lack of data for other European regions;
- uncertainty in questionnaires, as the participants estimated frequency of consumption without an instruction on portion sizes;
- portion sizes for the Czech population were not available for all food items and were roughly estimated based on a literature search.

3.3.3 Validation

As mentioned above, although the workflow is prototyped in R, the final implementation will be integrated into MCRA. The workflow will be validated by comparing the MCRA outputs to those of the R script, as well as through unit and regression testing, following the approach described in section 3.1,

Validation of the methodology implemented within the workflow can be characterised by a self-validating nature. As mentioned in D8.4, due to absence of authentic PFAS EDIs to compare with (“ground truth”), a validation process poses a challenge. However, a comparative analysis of derived individual EDIs based on the Czech population data has been done and could be considered as a self-validation. As seen from the Demonstration section 3.3.2 and the

limitations stated above, only dietary exposure was included for EDIs estimates from external exposure. Thus, we didn't expect the EDIs derived from dietary exposure (external EDIs) to closely match the EDIs derived from blood levels (internal EDIs). This is because external EDIs represent only one part of internal EDIs, which also includes other exposure sources such as dermal and inhalation. From this point of view, we obtained relatively satisfying preliminary results for PFOS since all Czech adults had higher internal EDIs. However, different results were obtained for PFOA as for one third of the people external EDIs estimates exceeded internal EDIs. Since the application is illustrative and has limitations, it should be interpreted with caution and accounted for further methodology development and input data accuracy.

Inconsistent error bars for external preliminary EDIs assessed based on the analytical method need more investigation and comparison with the output from a more computationally demanding MC simulation.

An additional way of workflow validation could be to perform MCRA using HBM4EU European population data that is foreseen to become available at the end of Y3 - beginning of Y4. At the starting point a comparative analysis of external dietary exposure can be performed, using the MCRA tool and R that is used currently for the workflow application. It will require the following actions:

- 1) Comparison between the external exposure model used in MCRA and the model currently implemented in R, including opportunities for alignment, major differences and similarities.
- 2) Uploading the PFAS occurrence data currently used in the case study workflow into MCRA with the aim of using the same input data.
- 3) Estimating external EDIs using the MCRA tool on the one hand and the current R environment on the other, followed by a comparison of the results.

After validation of the external EDIs, the same step can be performed for internal EDIs using a PFAS PBK model deployed in MCRA, and the adapted model developed by T6.2.2 team.

3.3.4 Next steps

The model for external exposure assessment was developed and will be applied on HBM4EU adolescent data from other European countries to generate PFAS EDIs based on dietary patterns and PFAS occurrence data. Additionally, it will be possible to align the developed exposure model with the MCRA exposure model and deploy selected uncertainty methods (such as MC simulation method, analytical method paired with the Fenton-Wilkinson approximation) in MCRA to evaluate the predictive uncertainty of exposure models.

Once the PBK model and the parameterisation are available, the workflow will be tested on HBM4EU adolescent data using global sensitivity analysis to identify the most influential parameters, such as eFAST. Additionally, MH-MCMC will be used for parameter calibration, followed by a comparison of modelled exposures. The developed uncertainty assessment methods will be applicable for various PBK models and compounds. Further on, uncertainty assessment methods will be suggested for other types of models and domains (see section 4.2). To reach this goal, partners will be requested to provide brief information regarding uncertainty methods they use in their types of models/domains. Developed methods for uncertainty assessment may be further implemented in MCRA thereby making them available to more potential users.

3.4 Environmental burden of disease assessment

Burden of disease (BoD) analyses quantify the current health status for countries or specific population groups. Environmental BoD assessments focus on attribution of disease burden to specific risk factors, to help guide health policy decisions. Despite the clear public health significance of chemicals, quantitative studies assessing their impact on population BoD are limited or outdated [22]. PARC T6.2.4 focuses on developing methodological procedures for estimating the environmental BoD (EBD) resulting from exposure to chemicals in European countries and provide case studies for selected chemicals and population groups.

The goal is to develop a workflow for EBD assessments within the PARC model network that is adaptable across **population, substances, health effect, and BoD indicators**. However, methodological approaches can vary significantly depending on the context and data availability. Further harmonisation of both methodology and data inputs is necessary. Therefore, the workflow is being developed in an iterative fashion. This Deliverable presents

an initial prototype for EBD assessments, following the methodology previously applied to assess the impact of pyrethroid exposure on ADHD effects [23]. The approach aligns with the steps for comparative risk assessment outlined in [24]. To estimate the proportion of disease burden attributable to exposure to a specific substance (risk factor), the population-attributable fraction (PAF) must be calculated. This requires an estimate of exposure within the relevant population, along with a relative risk (RR) or comparable effect measure. In the prototype workflow, Levin's equation is used to estimate the PAF. The attributable BoD is then obtained by multiplying the baseline burden of disease (sourced from databases such as the IHME database¹⁶) with the PAF [24].

The workflow is implemented in MCRA to facilitate future EBD assessments based on measured exposures (HBM-based workflow in section 3.1) and modelled exposures (AEA workflow in section 3.2).

3.4.1 Description

The workflow integrates data and models across three key domains of the conceptual framework (Figure 1): human biomonitoring, epidemiology and risk assessment / EBD. Over time additional domains may be incorporated, with a particular focus on exposure assessment and toxicokinetics. This would enable EBD assessments based on modelled exposures. The current prototype relies solely on measured exposures from HBM data. The MCRA-web platform serves as the central UI for applying the workflow.

As shown in **Erreur ! Source du renvoi introuvable.**, the primary input for the workflow is an individual-level HBM dataset. Therefore, the first steps of the workflow proceed analogously to what was described in section 3.1. The VITO HBM tools are used to convert the data to the PARC harmonised format. Subsequently, the data is processed as desired in MCRA, including optional imputation, normalisation, biomarker conversion and kinetic conversion steps. The output of module *HBM analysis* are acute or chronic exposure estimates at an internal level, or potentially at an external level following kinetic conversion. These are passed on to the module *Environmental burden of disease*.

The MCRA EBD module bins the empirical exposure distribution that was estimated in *HBM analysis*. Currently the bins are hard-coded as P0-P5; P5-P10; P10-P25; P25-P50; P50-P75; P75-P90; P90-P95; and P95-P100. In later versions of the workflow, binning options will be made available as user input. After binning, the PAF is estimated using Levin's formula. For this purpose, an exposure-effect function is used which is input as data via module *Exposure effect functions*. The data format allows for specification of a wide range of functions and different effect measures including RR and odds ratio's (OR). Finally, the attributable burden is estimated by multiplying the PAF by the baseline disease burden. Currently, the baseline burden is hard-coded in the workflow. In the next versions, the baseline BoD information will be entered as data by the user in the module *Burden of disease data*. The BoD data can be extracted from different sources, and over time automatic connections to some of these databases may be considered. Finally, the attributable BoD is displayed in a table together with the calculation steps followed. In the future, if desired, the BoD can be reported per 1000 000 individuals in the population. For that purpose, the user can input population characteristics via the module *Populations*.

3.4.2 Demonstration

As an initial demonstration of the workflow, synthetic data was generated based on an EBD assessment of ADHD effects associated with pyrethroid exposure. Following the approach of Purece et al, the example utilises urinary 3-phenoxybenzoic acid (3-PBA)-levels observed in French adults between 2014 – 2016 [23]. This compound serves as a non-specific biomarker for exposure to several pyrethroids. First-morning concentrations for this biomarker were extracted for several percentiles from the European HBM dashboard¹⁷. Subsequently, individual-level HBM data was simulated based on these percentile values, as the MCRA module *HBM concentrations* requires individual-level input data. Finally, the PAF was estimated based on a log-linear model of the ORs used in Purece et al. [23].

Erreur ! Source du renvoi introuvable. displays the UI for the EBD workflow in MCRA. The yellow section in the left-hand menu highlights the main EBD calculator module, which allows the user to configure the assessment.

¹⁶ https://ghdx.healthdata.org/ihme_data

¹⁷ <https://hbm.vito.be/eu-hbm-dashboard>

Additionally, the menu includes a (green) data module for submitting exposure-effect functions. The figure also showcases the main output of the

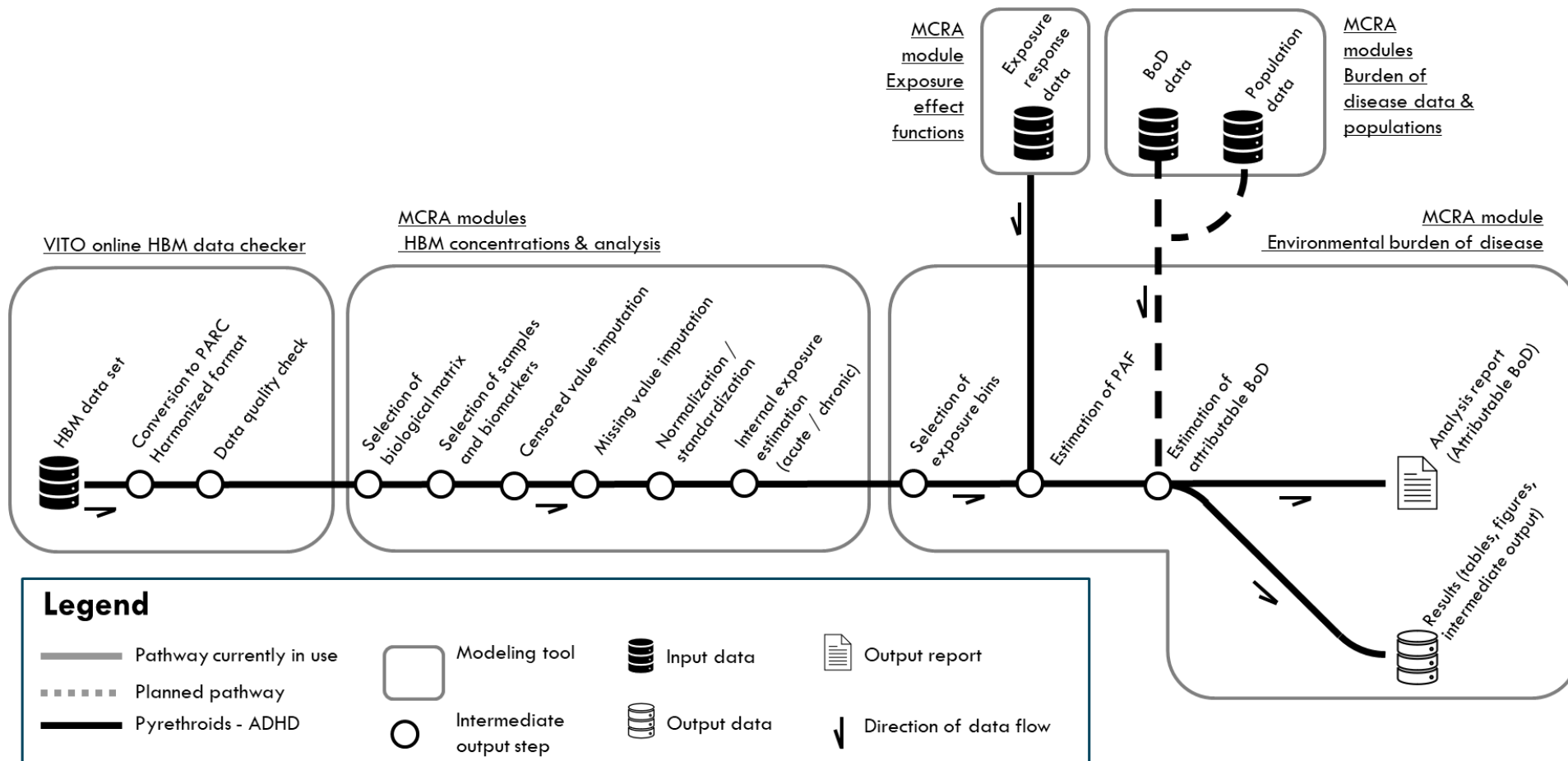


Figure 9 Schematic display detailing the workflow for environmental burden of disease assessment, emphasising the data, modelling steps and software tools used.

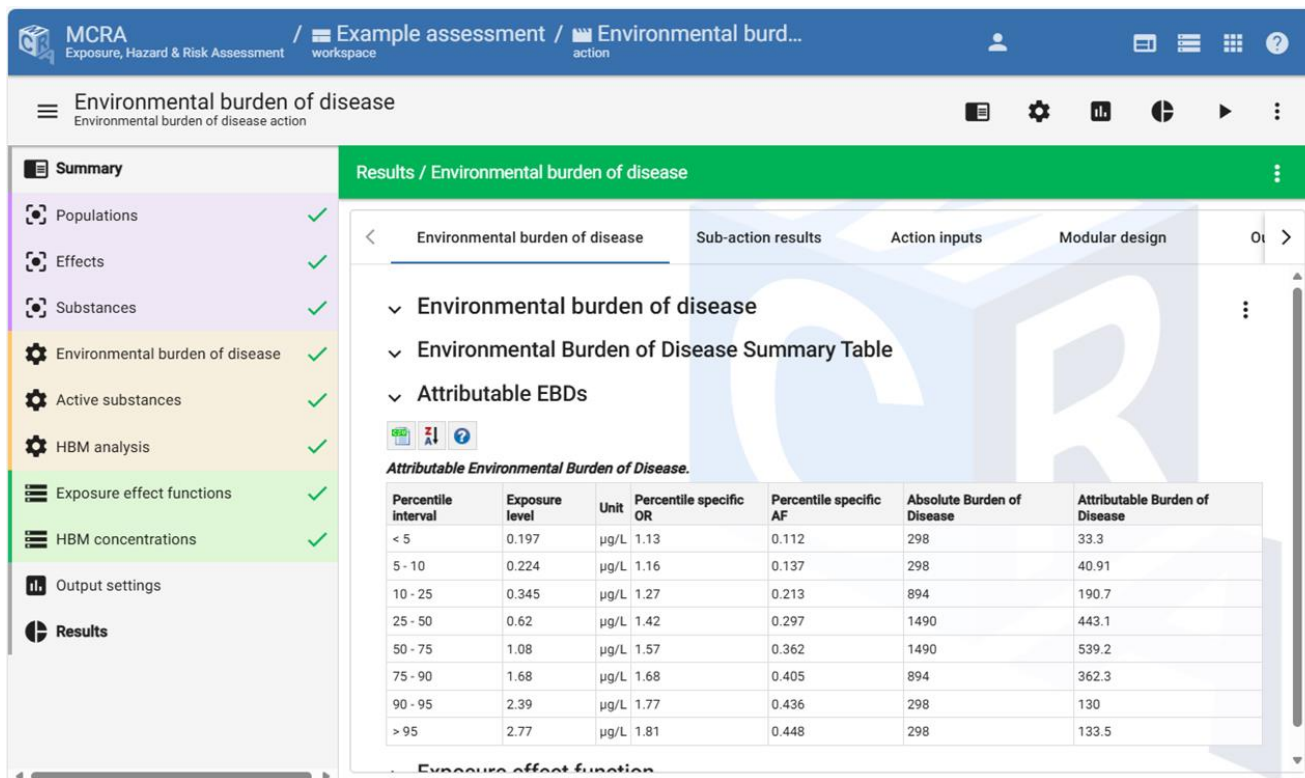


Figure 10 Example of the user interface for the environmental burden of disease assessment workflow, displaying an output table with attributable burden of disease values expressed as disability-adjusted life years

current EBD implementation: a table detailing the attributable BoD for each exposure bin. Future iterations of the workflow will offer a more expanded output, including an estimate of the total attributable BoD.

3.4.3 Validation

At present, the workflow is validated using the results from case studies in T6.2.4. As the workflow becomes more mature, a similar validation approach will be followed as in section 3.1.

3.4.4 Next steps

It is the intention that this proof-of-principle will be expanded into a more functional EBD toolbox. For this purpose, a functional design for the toolbox is currently being developed together with T6.2.4 partners. Initially, developments will focus on PAF-based calculations, supporting different BoD indicators and the use of measured and modelled exposure estimates (HBM and AEA workflows).

3.5 QSAR-based chemical grouping

Cumulative exposure and risks assessments are commonly performed on groups of chemicals, in which hazard-driven criteria are used for grouping. QSAR models to predict compound activity regarding molecular initiating events (MIEs) and upstream key events (KEs) of established adverse outcome pathways (AOPs) can be used to assist in this grouping for large numbers of chemicals. The aim of this workflow is to assess for some adverse (health) effect of interest (e.g., thyroid disruption, endocrine interference) those chemicals from some given (large) set of chemicals from a specific class of interest (e.g., pesticides), that (may) cause this effect of interest. Trained QSAR models generate activity estimates for chemicals based on upstream MIEs and early KEs of AOPs and AOP networks, which are then used to predict downstream activity on AOs. This process supports the prioritisation and grouping of chemicals for RA and decision-making.

3.5.1 Description

The QSAR-based grouping workflow is built as a modular pipeline that integrates chemical structure information, predictive QSAR modelling and established AOPs to estimate the likelihood of health effects associated with chemical exposure, therewith exploiting mechanistic understanding of how chemical interactions at the molecular level can lead to adverse outcomes (AOs), as captured in AOP networks (AOPNs). It starts with the selection of a specific chemical class (e.g., pesticides) and applies existing QSAR models implemented in the VEGA platform (version 1.2.4, www.vegahub.eu) to predict molecular features useful to characterize the substances, such as structural alerts, molecular initiating events (MIEs) or key events (KEs) relevant to establish adverse outcome pathways (AOPs). The VEGA software includes collections of these features, but there are several others. Furthermore, it is possible to use in silico-models for endocrine disruption endpoints. The complete list of the models used in this case study is indicated in paragraph 3.5.2. The output consists of probabilistic activity estimates (e.g., active, inactive, possible active, possible inactive) for each compound at various mechanistic stages. These predictions are then fed into the MCRA software, where they are integrated with the corresponding AOPN. Using a Bayesian approach, activity scores are propagated from the MIE/KE level up to the AO, providing a hazard-based group membership estimate. This enables users to screen, prioritize, or assess chemical risks depending on the objective of the assessment. The workflow is flexible, allowing multiple purposes and output types (e.g., binary classification or probabilistic ranking), and can adapt to future developments such as the inclusion of new models or refined AOP ontologies.

The workflow may be used for different purposes. Depending on the purpose, different criteria apply. Often assessors use the term problem formulation for this. We have identified at least these cases:

- **Use for classification:** The preferable scheme is to refer to classification according to official definitions, such as GHS/CLP. In this way substances are grouped into classed, such as PBT, or CMR, etc.
- **Use for prioritisation:** Prioritisation provides an order within a certain list of substances, while in the screening and classification procedure all the substances with the same membership are equal. The order can be defined based on the potency (preferably) or the reliability of the outcome. For instance, it may be of interest to sort them, to prioritize the substances to perform experimental test on those of the highest concern. For this purpose, the software JANUS was developed, proceeding beyond the simple classification.
- **Use for risk assessment:** The assessment requires much more reliable data. The processes described above are in most of the cases preliminary activities, and further refinement follows. In the case of the assessment, the decision is taken. Thus, a sound evaluation of the values is necessary, gathering multiple lines of evidence. Furthermore, it is common that the assessment is done for RA, and in this case a quantitative value is needed. Conversely, for the other purposes, a classifier is sufficient.

Ideally, the architecture is the same, using the same models, but finetuning is applied depending on the purpose. Ideally, multiple conditions can be run by the software system, offering multiple overall outcomes results to the user, which will have an overall view depending on the conditions.

The workflow links data and models from in the domain of hazard characterisations (see Figure 1). In this workflow (see Figure 11), QSAR activity predictions were obtained by the VEGA software and they can be associated with specific MIEs and KEs of an identified AOP, for instance for endocrine disruption, in the case that experimental data are not available. These activity estimates are provided to the MCRA module *active substances* (or, assessment group memberships), where they are linked with MIE/KE relationships from the AOP (or AOP network) of interest. Next, still within the MCRA module *active substances*, the activity scores at the level of MIE/KE are aggregated (or propagated) to an activity estimate at the level of the AO, therewith forming the group membership estimates for hazard-based grouping. For aggregating/propagating activity estimates of upstream KEs/MIEs to the level of the AO, different calculation models are available in the MCRA software (see documentation¹⁸).

3.5.2 Demonstration

As an initial demonstration of the workflow, we are addressing estrogen receptor-, androgen receptor-, thyroid- and steroidogenesis-mediated endocrine disruption. A list of 214 pesticides was selected, for which SMILES (simplified molecular input line system) codes were retrieved using a semiautomated workflow developed in

¹⁸ <https://mcra.rivm.nl/documentation/10.1.4/modules/hazard-modules/active-substances/active-substances-calculation.html>

KNIME [33]. Using the VEGA software¹⁹ (version 1.2.4), activity of the selected pesticides was predicted applying the following QSAR models:

- Estrogen Receptor-mediated effect (IRFMN-CERAPP) (version 1.0.1)
- Estrogen Receptor Relative Binding Affinity model (IRFMN) (version 1.0.2)
- Androgen Receptor-mediated effect (IRFMN-COMPARA) (version 1.0.1)
- Thyroid Receptor Alpha effect (NRMEA) (version 1.0.1)
- Thyroid Receptor Beta effect (NRMEA) (version 1.0.1)
- Glucocorticoid Receptor (OBERON) (version 1.0.0)
- Thyroperoxidase Inhibitory Activity (OBERON) (version 1.0.1)
- Endocrine Disruptor activity screening (IRFMN) (version 1.0.0)
- Steroidogenesis (OBERON) (version 1.0.0)

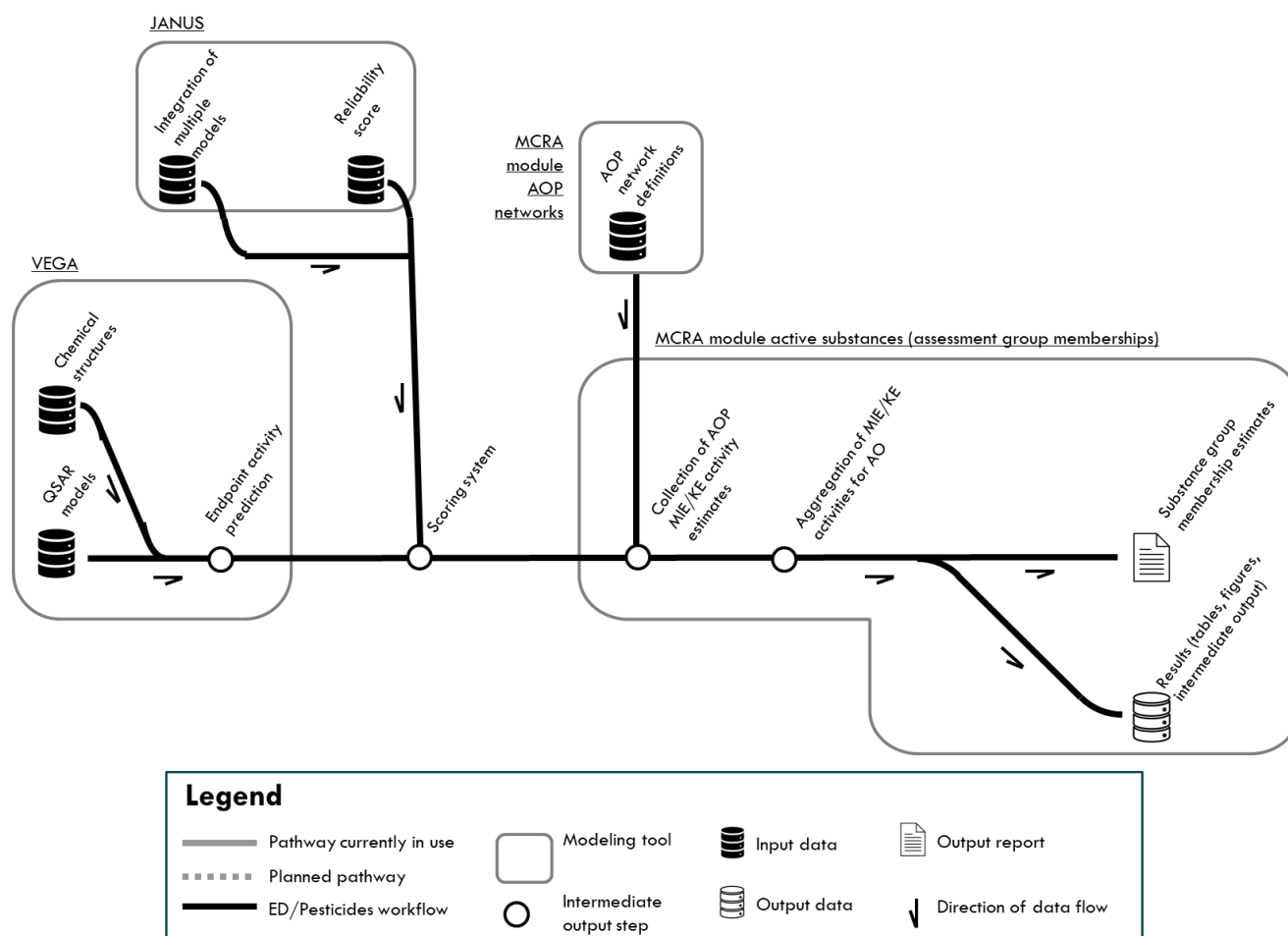


Figure 11 Schematic display detailing the workflow for QSAR-based chemical grouping, emphasising the data, modelling steps and software tools used.

Within each mechanistic domain, different situations can be identified. For thyroid and steroidogenesis, if one of the models is positive, it is sufficient to assign an activity label to the substance. Indeed, there are multiple pathways producing the final outcome, which is achieved when at least one pathway is active. Conversely, in the cases of estrogen and androgen, the models must be considered in a mediated approach, requiring the majority of the predictions to declare the substance active. Indeed, the different models refer to the same pathways, even if they cover one or more steps in the same pathway. For instance, the simplest model simply refers to the MIE. Ideally, all models should agree (unanimity). When unanimity is not achieved, the reliability of the assessment decreases. In this context, JANUS²⁰ provides valuable support through its pre-implemented scheme for estrogen and androgen

¹⁹ www.vegahub.eu

²⁰ www.vegahub.eu

evaluation. To ensure the proper integration of models for these endpoints, we can rely on the tool's algorithm, which considers not only the predicted values but also the reliability of the predictions. This approach translates the assessment into a well-defined and validated scoring system, enhancing clarity.

The predictions provided by VEGA are then translated to facilitate a group membership assessment. In some of the models, the memberships is not simply binary (active or inactive). In these cases, the following scheme was adopted, interpreting the numeric values as probabilistic estimates of activity:

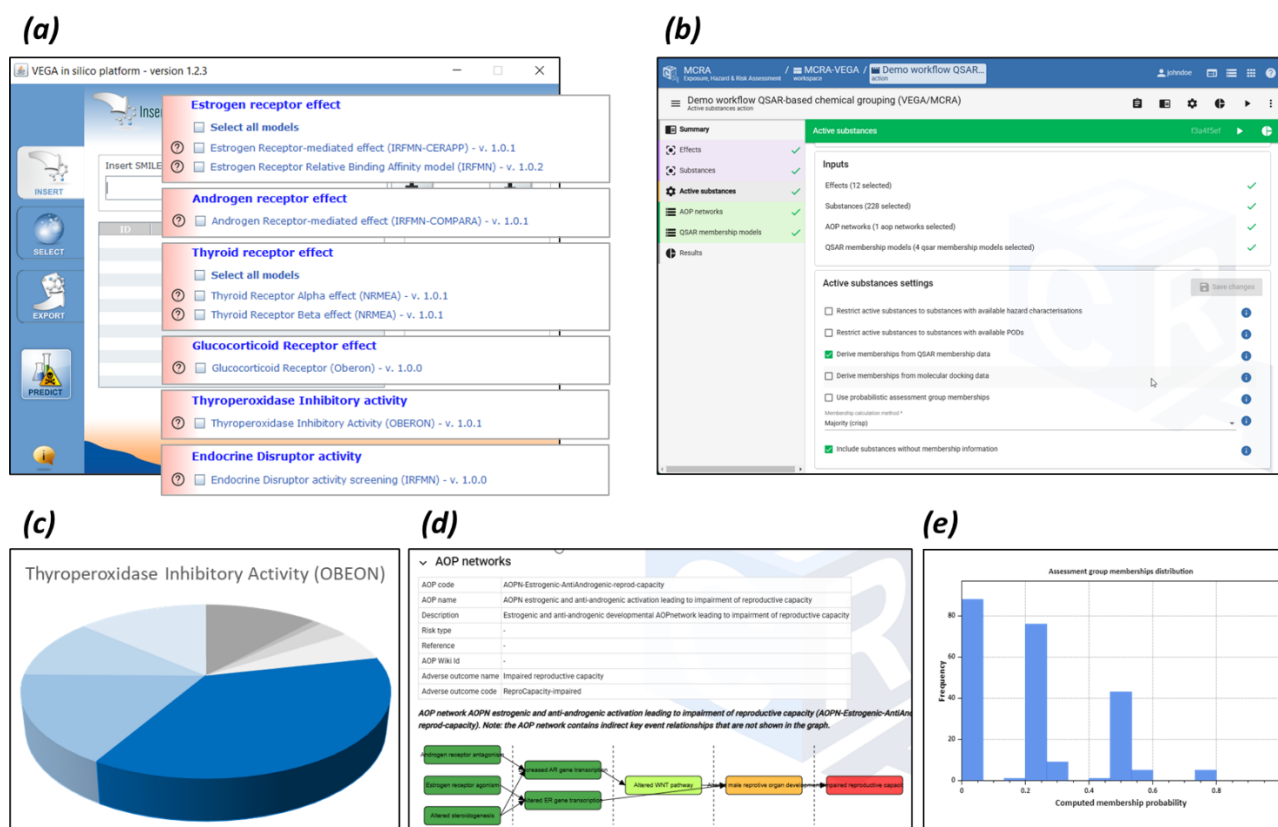


Figure 12 Example of (a) the user interface of the workflow for QSAR-based chemical grouping, displaying the VEGA user interface used to obtain QSAR model predictions for the selected chemical structures (pesticides); (b) the MCRA user interface for deriving assessment group memberships; (c) a pie chart of the activity predictions by VEGA on one of the selected endpoints; (d) the AOP network section of the MCRA output report, summarising the linked AOP network; (e) a histogram of the group membership estimates for the AO.

- Active = 1
- Inactive = 0
- Possible active = 0.75
- Possible non-active = 0.25

The QSAR-based activity predictions are provided as data to the MCRA module *active substances*. These activity estimates at MIE/KE level are then aggregated/propagated to AO-level assessment group membership estimates using a probabilistic (Bayesian) approach proposed by Kennedy et. al [34]. It should be noted that this demonstration represents a proof-of-principle only. Considering an AOP analysis, we identified some models more related to the MIE, such as those where the binding affinity with the receptors is explicit, and others closer to the final adverse effect or representing the adverse outcome, such as the Endocrine Disruptor activity screening (IRFMN) (version 1.0.0), which covers the apical effect. In this way, different in silico models can cover data gaps related to the information processed within the AOP: some models are related to the MIE and others to the apical effect. This analysis helps to start building an AOP network using the abovementioned models.

3.5.3 Validation

The workflow and methodology are still in an early stage of development, and no specific validation has been done. For the validation, we need to collect external data not used in our training set. The validation step will be done once the workflow is completed using new data. To this end, we will collect external data from reliable sources, ensuring that these data fall within the applicability domain of the model and represent a sufficient diversity of chemical structures and biological responses. Where possible, we will aim to include a balanced representation of both positive and negative outcomes. The description of the models is done using the [4] QSAR Models Reporting Format (QMRF), a harmonised template for summarising and reporting key information on QSAR models, including the results of any validation studies. The QMRFs of the models listed in the paragraph 3.5.2 are freely available on the VEGAHUB website²¹. The models have been developed and tested on external validation sets. Further validation will be done within PARC.

3.5.4 Next steps

For the next steps, a more appropriate AOP network needs to be identified as a test case for building up the methodology, starting from the data provided by the VEGA models and exploiting the information already available in the AOP-Wiki system as well as by exploiting the results obtained in other tasks of PARC where work on AOP for endocrine disruption is under development, in particular in WP5. Already available AOP workflow (as for example those for estrogen or androgen mechanism) serves as valid and consolidated scheme to concretize the case study. In addition, IRFMN is working within other projects developing more in silico models for endocrine disruption. Thus, in the near future more models will be available, and this can represent the possibility of further improvement within the scheme that we have defined, adding new models and improving the confidence on the overall results.

For further development of the workflow, the following model refinements have been identified:

- Usually, the uncertainty associated with predicting the MIE is lower compared with that for apical effect. On the other side, often there are more data on the initial assays, and less on the apical effect.
- It is possible to imagine a conceptual scheme with weights assigned to the outcome of each building block: MIE, KE, etc. The relevance of the outcome increases towards the AO. The reliability of the outcome may decrease towards the AO.
- The weights assigned to each building block may be assigned based on the relevance and reliability of the individual outcomes of each step, i.e. on the results of the models predicting them. The reliability can be measured based on the predictivity, using statistical methods. The relevance must be assigned arbitrarily. As an alternative, a Bayesian strategy can be applied, if there are sufficient experimental data with the same chemicals for the separate steps.
- The models should be distinguished, if they provide a quantitative outcome or a class. In the case of VEGA, for ED, classifiers are the common case.
- VEGA provides info on the reliability of the prediction, and this value should be used together with the prediction. In the JANUS software, those values are combined into an overall final score.
- This workflow clearly benefits from comprehensive, consistent, and FAIR databases for AOP networks. Adoption of harmonised terminology/ontologies for identification of the different building blocks of AOP networks (e.g., MIEs, KEs, and AOs) is essential. For further development of the workflow, it is essential to also connect to development of an AOP ontology, currently being done in PARC WP7.

3.6 Environmental risk assessment

3.6.1 Description

The STOP framework is an integrative and conceptual workflow designed to link chemical releases from their sources to their potential biological effects, providing a comprehensive approach to understanding the cascading impacts of environmental contaminants. It's envisioned that the STOP would be chemical-agnostic, i.e. not limited to one group of chemicals, but more generally applicable to a large chemical domain. From a pragmatic point, focus will be given to selected groups of chemicals identified to be relevant for selected case studies in PARC (i.e. in WP5 – bisphenols and PFAS; T6.1.2 - legacy metals and inorganic relevant from Arctic exposure scenarios; T6.4.4 –

²¹ <https://www.vegahub.eu/portfolio-item/vega-qsar-models-qrmf/>

Mixtures of PPPs), to demonstrate utility and deploy an iterative handling and testing of the framework and workflows (modules). This framework integrates the conceptual framework of the aggregate exposure pathway (AEP) with the AOP.

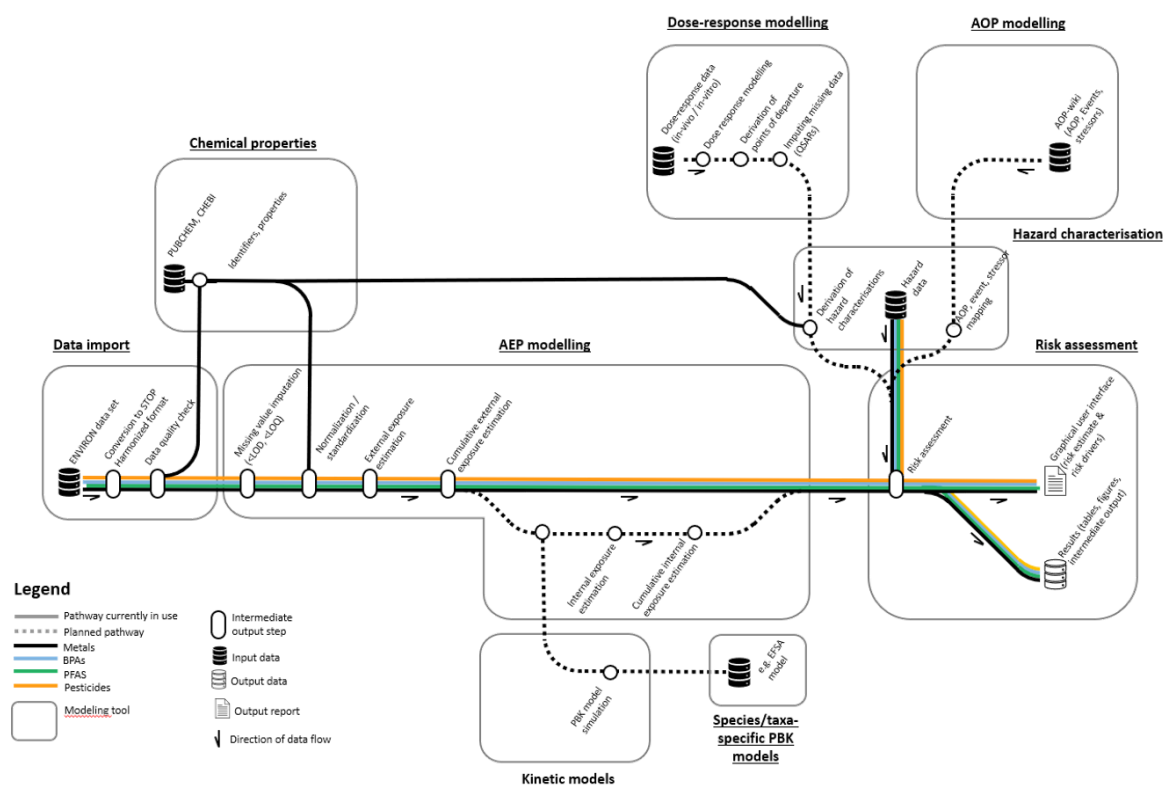


Figure 13 Source-To-Outcome Pathway (STOP) architecture and data flow.

Integrating the AEP and AOP frameworks into the STOP is essential for creating a holistic view of chemical exposure and risk. By incorporating quantitative AEPs, we can capture comprehensive exposure scenarios relevant to environmental species. Meanwhile, quantitative AOPs provide insight into the biological consequences of these exposures across multiple levels of biological organisation. This integration allows for more precise and informed risk predictions. A combination of predictive approaches such as QSAR models, Quantitative Structure-Property Relationship (QSPR) models, alongside established toxicity thresholds and DR modelling using experimental data from existing databases and in-house studies, plays a crucial role in this infrastructure (Figure 13). These tools will collectively aid in identifying pollution hotspots, risk drivers, vulnerable species, key mechanisms of toxicity, and assess potential cumulative impacts at the individual, population, community and ecosystem levels.

A number of tools and approaches are envisioned in an integrated way to form the STOP infrastructure, ranging from data-hosting databases, to dedicated analysis and visualisation dashboards and UIs. Exposure and effects data are stored in NIVA’s Risk Assessment database (Radb), an ORACLE database designed to support a comprehensive workflow from source (exposure) data, through HC to the prediction of risk. A combination of use/reuse of existing data and specialised import functionality for chemicals, exposure and hazard data ensure a flexible infrastructure for handling heterogeneous data.

3.6.2 Demonstration

Demonstrations to date focus on applying individual models and tools within the STOP framework to specific use cases. These efforts illustrate the functionality and utility of separate modules in the following areas:

Exposure assessment: A common data conversion pipeline has been developed in R to facilitate environmental exposure data extraction to a FAIR, standardised format and import to Radb. The effort has been a joint venture with PARC P7.2.2’s Chemicals in the environment metadata schema. A dedicated pipeline has been adapted to

Norway's principal governmental exposure datasets ([Vanmiljø](https://vanmiljo.miljodirektoratet.no/)²², [JOVA](https://www.nibio.no/tema/miljo/jord-og-vannovervaking-i-landbruket)²³, etc.) and used to convert 30+ years of aquatic monitoring data collected across Norway. Additionally, an RShiny app-based tool (eData) is under development to aid users in converting novel exposure datasets into a FAIR format for subsequent use, archiving or import to RAdb. These two tools allow a more targeted approach for both small and large datasets, exemplified by exposure data from PPPs in a Norwegian stream ([Erreur ! Source du renvoi introuvable.](https://stop-q-data.p.niva.no/)).

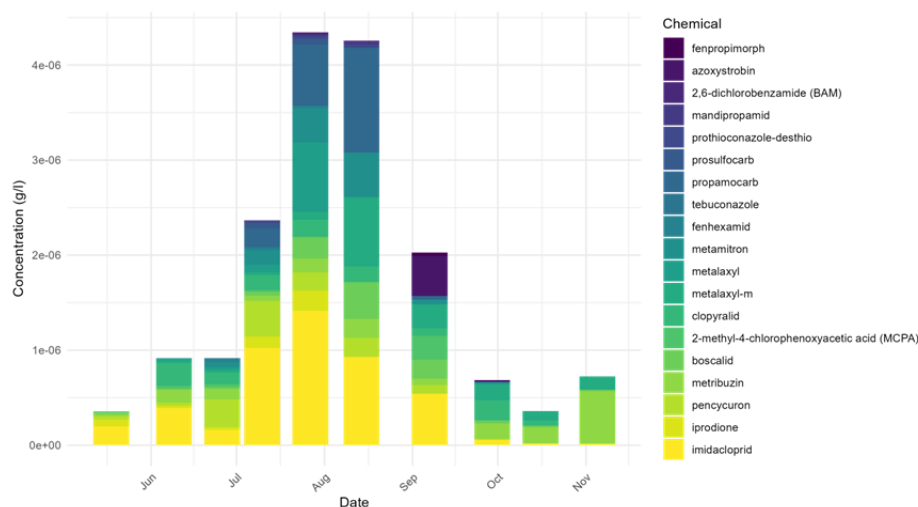


Figure 14 Measured concentrations of different PPPs in a Norwegian stream receiving complex emissions from agricultural activities.

Dose-response modelling: Specific attention has been given to ecotoxicological DR modelling for PoD estimation and quantitative AOP development. A dedicated UI or dashboard for DR data (qData) has been developed as a FAIRification hub for (eco)toxicological DR data in collaboration with WP5 and WP7.2. The qData UI²⁴ enables detailed analysis of PoD estimations such as benchmark concentration (e.g. BMC10 or BMC50), no-observed-effect concentration (NOEC), or the no-significant-effect concentration (NSEC)[37] Resulting PoD estimations are envisioned to expand on available ecotoxicological data and support more complex hazard assessments such as species sensitivity distribution (SSD) for use in chemical RA and environmental quality assessments ([Erreur ! Source du renvoi introuvable.](https://stop-q-data.p.niva.no/)).

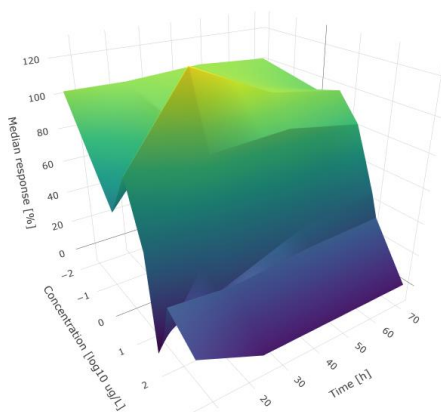


Figure 15 Concentration time response modelling for the PPP diuron in freshwater algae. The data has been reported in a standardised format in the quantitative dose-response modelling dashboard qData and time-dose response relationships were modelled using R and R packages drc and bmc.

Hazard characterisation: Hazard assessment builds on all available data for a chemical of interest that is relevant to a given protection goal (i.e., to the individual, population or community). However, data sparsity is a prevailing

²² <https://vanmiljo.miljodirektoratet.no/>

²³ <https://www.nibio.no/tema/miljo/jord-og-vannovervaking-i-landbruket>

²⁴ <https://stop-q-data.p.niva.no/>

problem that needs to be handled in a tiered fashion. First of all, we are integrating major sources of publicly available ecotoxicity data to facilitate combined use of different data sources for RA. Additionally, we use these data to facilitate extrapolation to data-poor chemicals and biological species. Major public collections of experimental ecotoxicological hazard data include among others EnviroTox database²⁵ [38], the NORMAN Ecotoxicology Database [39, 40], and ECOTOX. ECOTOX is the largest publicly available source of ecotoxicity data from literature reviews, transparently and systematically collected and curated in a standardised and well-documented way [41] and it can be interfaced via an API or downloaded as a set of text files that can readily be turned into a database by users. Therefore, it is considered the most relevant compilation of experimental ecotoxicity study data for integration within the STOP. As the data is reported in differing ways both within ECOTOX and across other databases thereby hindering integration, we strive to harmonise the different data formats and create mappings between the different formats. In cases where data is sparse, uncertain or lacking, QSAR models from resources such as the VEGA hub will be used to fill data gaps. Figure 16 demonstrates the distribution of data for different PPPs in a Norwegian stream receiving agricultural runoff.

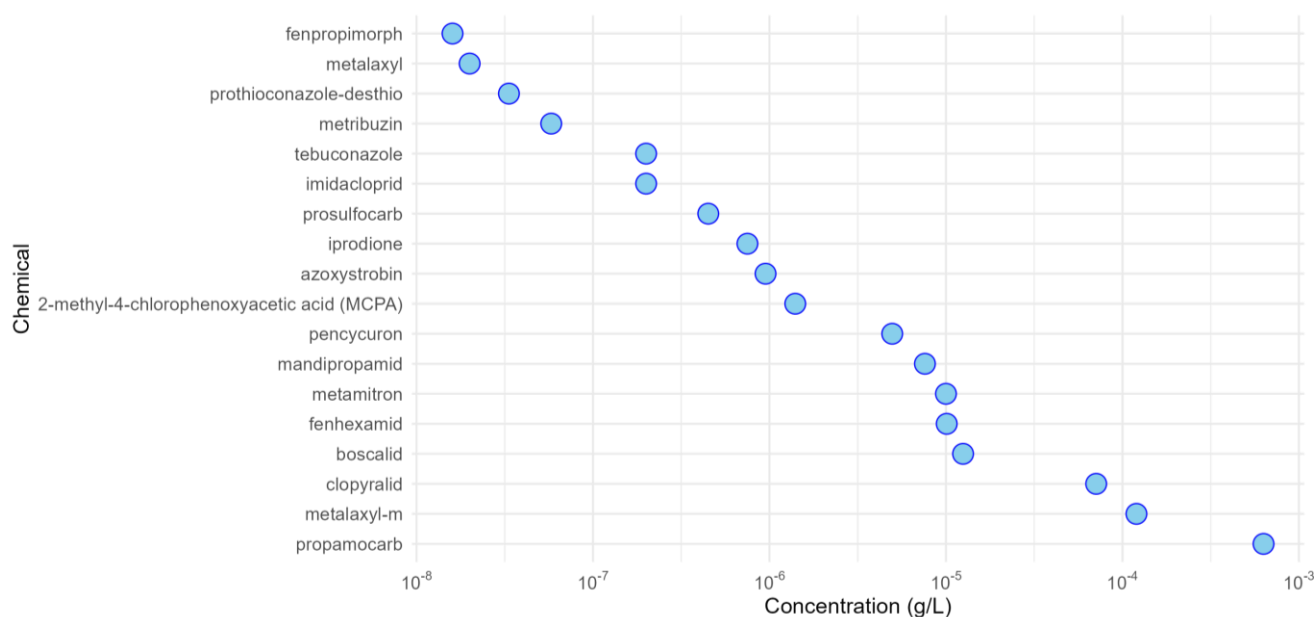


Figure 16 Ranking of the Predicted No-Effect Concentrations for different PPPs determined in a Norwegian stream receiving agricultural run-off.

Risk assessment: A key module in STOP is the risk assessment prediction, that relies on measures of chemical exposure (i.e. environmental concentration) and thresholds for no environmental impact. In these assessments, the risk quotient (RQ) is derived as the ratio between the environmental exposure concentration and predictions of environmentally safe thresholds, typically represented by the Predicted No Effect Concentrations (PNEC). The magnitude and frequency of exceedance of risk thresholds (RQ=1) is then used as an environmental risk metric. A similar approach is used for the combined (cumulative) RA, where the sum of risk quotients (SRQ) is indicative of the environmental risk a chemical mixture represents. Further refinement of the cumulative RA is achieved by considering species group or taxa-specific NOECs in cases where the SRQ exceed risk thresholds [42]. In cases where PNECs are lacking or uncertain, it is foreseen to estimate concentrations that are hazardous to no more than 5% of all species (HC₅) derived using SSDs [43]. An example of RA for different PPPs in a Norwegian stream receiving agricultural runoff is shown in Figure 17.

Integration and Visualisation tools: Initial prototypes of data visualisation dashboards and user-friendly interfaces have been explored to represent pathways and results from individual modules effectively. A prototype UI (STOPredictor) has been developed to facilitate end user-friendly visualisation of exposure, hazard and risk predictions performed in the STOP workflow (Figure 18).

These demonstrations showcase the potential of the STOP framework to provide insights at multiple scales, though they are currently limited to standalone models, databases and UIs rather than the entire integrated workflow.

²⁵ <https://envirotoxdatabase.org>

3.6.3 Validation

Validation of the STOP workflow is anticipated performed on selected case studies, typically representing environmentally-relevant freshwater and marine exposure scenarios. The exposure data sets have in many cases been organised and imported into RAdB through use of dedicated R scripts or will be submitted through use of eData prior to use in the STOP workflow. Concise validation beyond testing individual import, analysis or visualisation modules has not yet been undertaken.

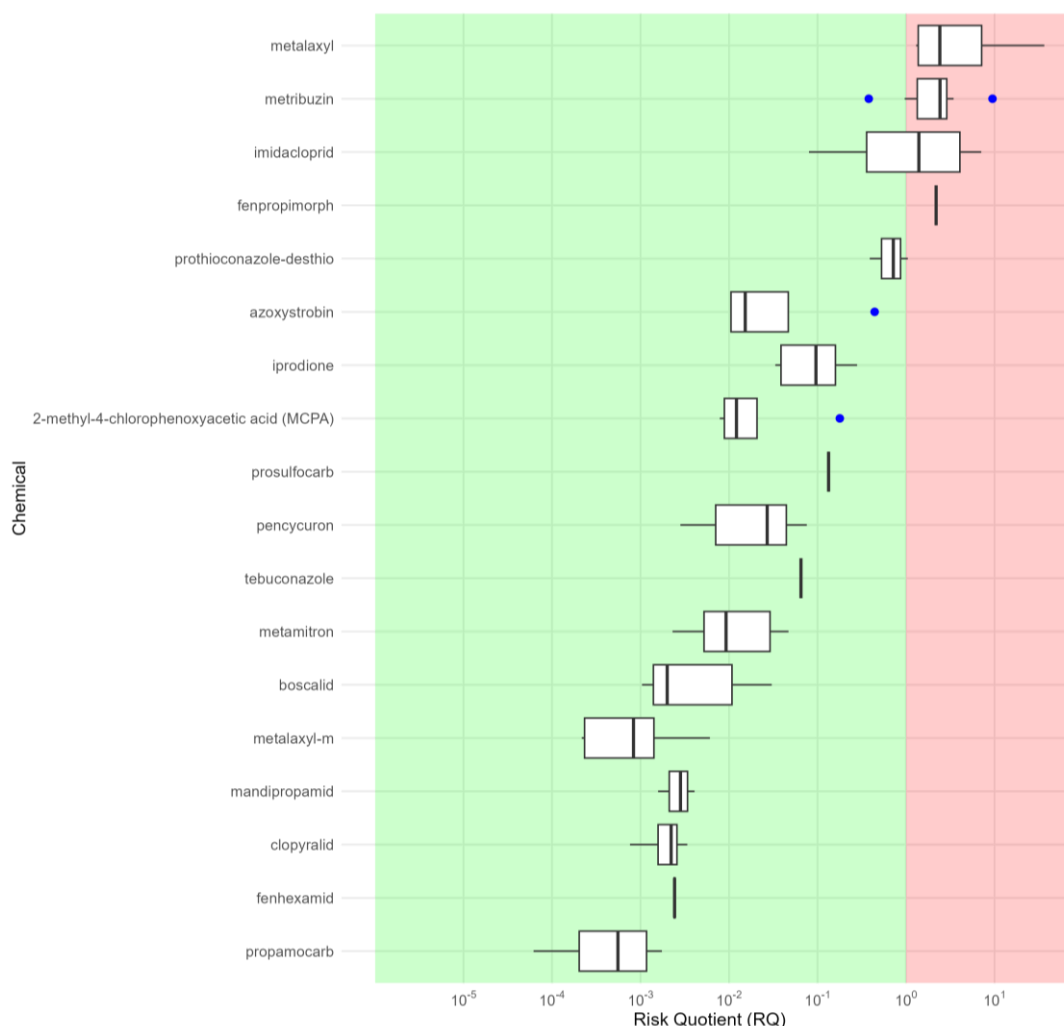


Figure 17 Risk Quotients for chronic exposure to PPPs detected in a Norwegian stream receiving agricultural runoff. The data show the median (vertical line), 25-75% quartiles (box), main data spread (horizontal line), and extreme values (blue dots). RQ values indicated in green ($RQ < 1$) are considered low risk, while RQ values > 1 , indicated in red, are considered a risk.

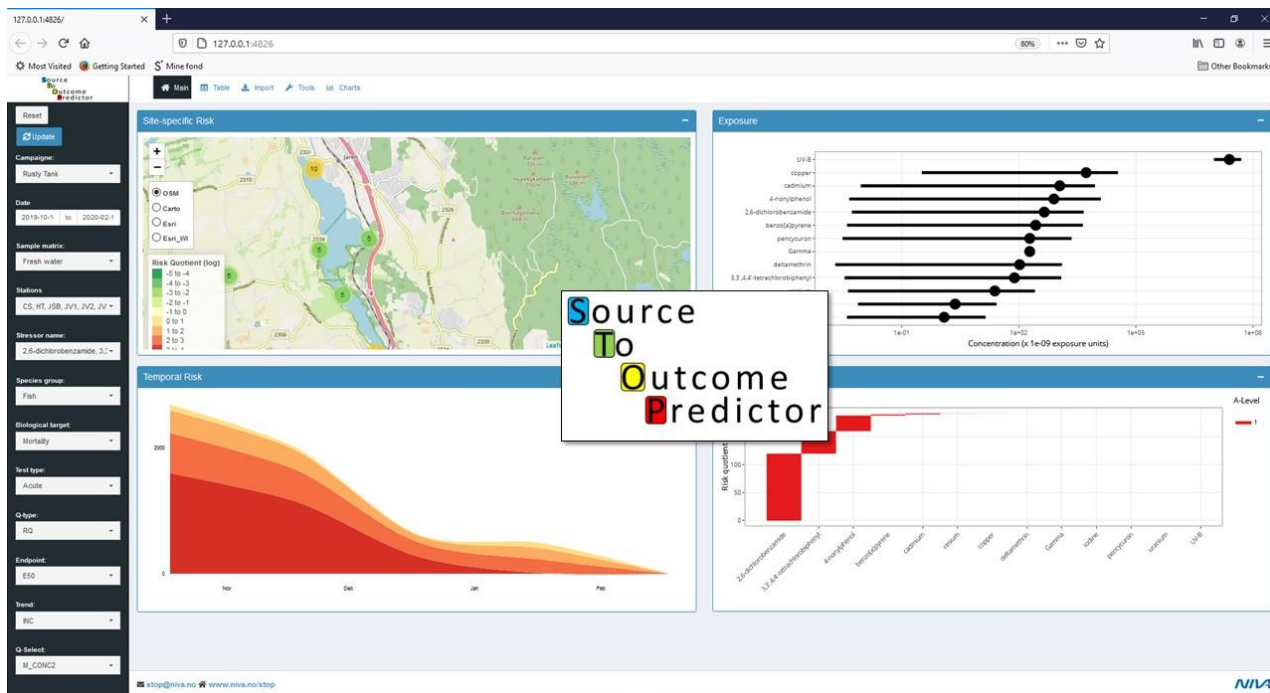


Figure 18 Prototype of the STOPredictor dashboard for visualisation of case study-specific data for exposure, spatio-temporal risk and risk drivers

3.6.4 Next steps

Work will continue to develop the component modules of the STOP framework. These will include:

- tData: development of an import and FAIRification module for ecotoxicological effect threshold data (tData) from DR-modelling (i.e. by qData) or user-defined data. Linkage with efforts to develop and use QSAR models would be a very relevant and logical next step in development.
- Aggregate Exposure Pathway: The tailoring of an integrated modelling framework for predicting flows of stressors from source to dose for relevant ecological targets (e.g. whole-body concentrations or concentrations in particular tissues of an organism). Although still in the scoping phase, use of off-the-shelf models and modelling tools to develop an AEP would be beneficial, but development of NAMs for AEP modelling may be required. Interfacing with efforts to develop the PARC FAIR PBK standards would be a relevant development for the next reporting period.
- AOP network analysis: Integrate AOPN analysis in the STOP workflow to development and refinement of an integrative approach for constructing and validating AOPNs. This includes leveraging qData and DR modelling to enhance the quantitative understanding of causal relationships between KEs. The approach will also support the expansion of quantitative AOPNs.
- Modules of the STOP will be tested in partnership with stakeholders across selected case studies, including Arctic marine, landfill leachate, and agricultural river pollution.
- Module integration work will continue in parallel with module development and testing, and a semi-automated data infrastructure will be prototyped and tested to minimise need for manual data transfer and conversion.
- Once modules have been developed and integrated to a satisfactory degree, work will begin to implement uncertainty and sensitivity analysis into the STOP.
- Finally, the fully integrated STOP will be implemented for one or more case studies. Effectiveness, ease-of-use, degree of automation, communication of outcomes and uncertainty will be assessed, first internally, and then externally via invited experts and peer-reviewed publications.

3.7 NAMs for risk assessment: The case of bisphenols

NAMs represent a paradigm shift in chemical risk assessment, offering ethical, efficient, and accurate alternatives to traditional animal testing, which raises ethical concerns, is resource-intensive, and may not reliably predict human health outcomes due to interspecies differences [44-46]. NAMs leverage advances in computational modelling (e.g., QSARs for toxicity prediction and PBPK models for ADME simulation among others) [47-49], high-

throughput in vitro systems (e.g., organ-on-chip, 3D cell cultures) [50-52], and human biomonitoring (HBM) [53] to align with the 3Rs principles (Replacement, Reduction, Refinement) [54, 55]. These approaches are further enhanced by machine learning, omics technologies, and probabilistic methods (e.g., Bayesian modelling) to refine exposure and risk estimates. In this study, we apply NAMs to assess bisphenols (BPA, BPS, BPF) using transcriptomics data from the OBERON project [56], which characterized their endocrine-disrupting effects in 2D and 3D cell models. The development of a computational pipeline linking gene expression changes to adverse outcomes (e.g., carcinogenicity, mutagenicity, reproductive toxicity) will allow to derive actionable insights for robust health impact assessments and decision making starting from molecular level. Additionally, we integrate exposure modelling and systems biology to construct adverse outcome pathway networks (AOPNs), providing a mechanistic basis for regulatory purposes.

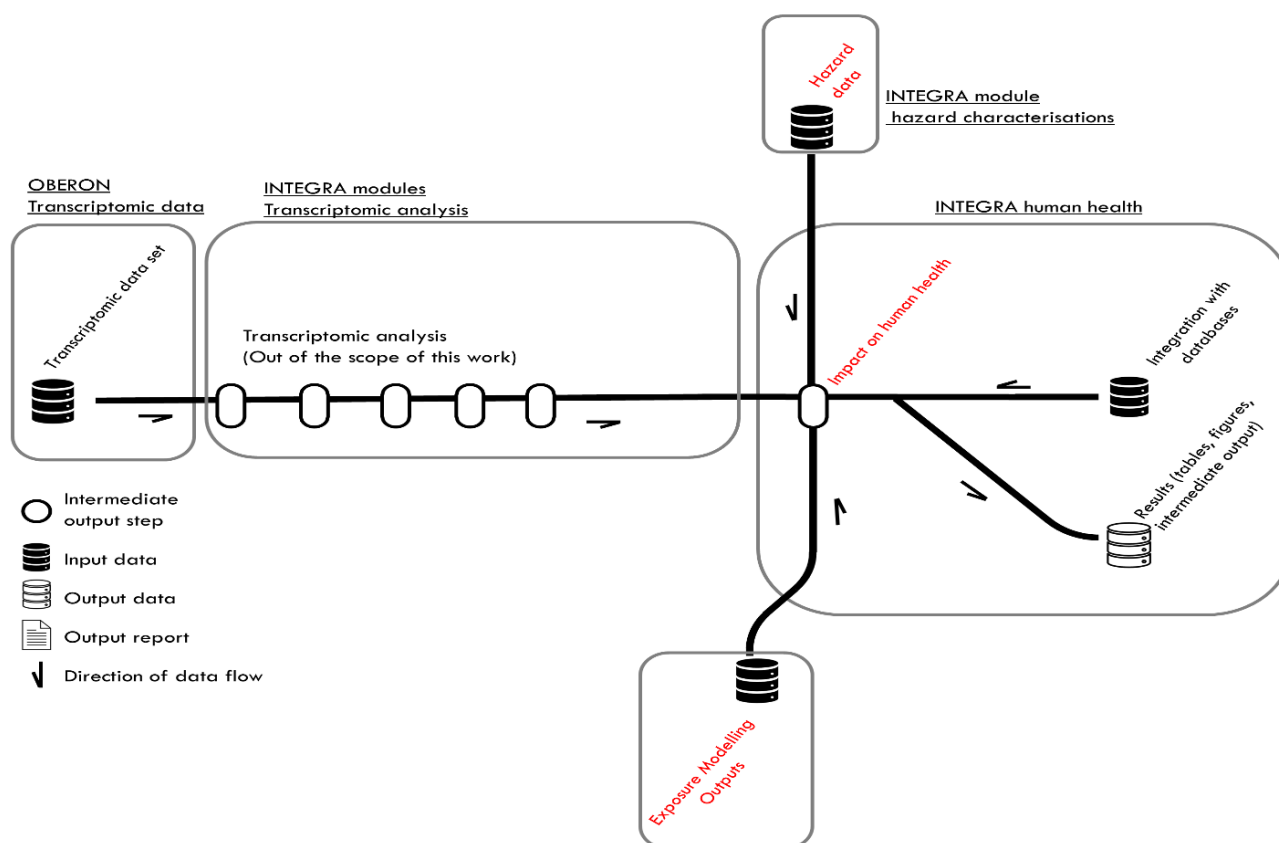


Figure 19. Schematic display detailing the workflow for NAM data assimilation in the bisphenols case study.

3.7.1 Description

The overall concept of the PARC model network is illustrated in Figure 1 and it has been discussed in detail in previous sections. The proposed workflow here is designed to systematically integrate two critical components of Figure 1: (1) the determination of internal exposure levels using established PBPK modelling frameworks as has already been done in previous Deliverables, and (2) the assessment of how these exposures impact human health. The PBPK modeling approach was based on the INTEGRA generic model framework. This model will be used to reconstruct exposure scenarios using reliable biomonitoring data (e.g., serum/plasma measurements) as well as estimation of the internal target tissue doses through validated physiological parameterization. The resulting dose estimates will inform the subsequent (toxicodynamic) assessments. Furthermore, the incorporation of advanced dose-response models will guarantee the association of internal estimates to adverse outcomes. This dual approach ensures a comprehensive evaluation of the EBD by linking exposure data to health outcomes. Figure 19 provides a visual representation of the overall conceptual framework, highlighting the interconnected nature of these components.

As illustrated, the workflow builds upon data analysed within the OBERON project framework as well as from an extensive literature review that is currently ongoing. While the detailed data analysis itself falls outside the immediate scope of this specific task, it is referenced here to provide context and ensure methodological completeness. The OBERON project has already conducted extensive omics data analysis, with findings currently being finalised in project publications that are in the advanced stages of preparation for submission. These publications will serve as a foundational resource for the subsequent steps in this workflow as well as to inform the basis of building dose-response models. Within the scope of Task 8.3, the workflow will focus on utilising the results derived from bisphenol analysis to evaluate the health effects associated with exposure. This will be achieved through the application of systems biology methodologies, which enable a holistic understanding of the biological mechanisms underlying exposure-related health outcomes. Systems biology approaches integrate multi-omics data (i.e., transcriptomic datasets, gene expression profiles, and metabolomic profiles) and computational modelling to elucidate the pathways through which exposure to bisphenols contribute to adverse health effects.

In alignment with Figure 1, the outcomes of this task will directly contribute to the broader assessment of the EBD. Specifically, the workflow will incorporate internal exposure dose estimations and transcriptomic data as key inputs, ensuring that the assessment is grounded in realistic exposure scenarios. These dose estimations will be derived from the models presented in previous Deliverables of Task 8.3 [2]. To further strengthen the analysis, hazard assessment methodologies will be applied. These methodologies will evaluate the potential risks posed by exposure to bisphenols, taking into account factors such as DR relationships, susceptibility across different population subgroups, and the cumulative effects of co-exposure to multiple environmental stressors. By integrating hazard assessment with systems biology and exposure modelling, the workflow will provide a robust and scientifically rigorous basis for quantifying the health impacts of bisphenol exposure. This workflow represents a systematic and integrative approach to assessing EBD, combining internal exposure modelling, systems biology, and hazard assessment methodologies.

The current assessment focuses on the following endpoints: carcinogenicity, mutagenicity, reproductive toxicity, and endocrine disruption.

3.7.2 Demonstration

As an initial solution to streamline the application of our methodology, we have developed an omics data template. This template is designed to enable users to efficiently transform their datasets into a format compatible with our analytical framework. By providing a standardized structure, the template ensures consistency and facilitates the seamless integration of diverse datasets into the workflow. A machine-actionable template for omics data is essential to standardize metadata collection, ensure interoperability, and enable automated data processing and integration across diverse platforms. The guidelines of the OECD's omics reporting framework, while useful for policy and high-level principles, lack the technical specificity and structured format required for computational implementation in omics research [58]. They are not designed to capture detailed, domain-specific metadata in a way that supports machine readability or automated workflows. At this stage, our focus has been exclusively on applications involving human genes, as this aligns with the primary scope of our research objectives. To ensure the reliability and robustness of our methodology during the development phase, we have employed artificial data for testing and validation purposes. Using human gene ENTREZ identifiers as our genomic anchor, we computationally generated an artificial transcriptomic dataset specifically designed for development and validation purposes. This synthetic resource was carefully engineered to maintain biologically meaningful gene-gene relationships while providing a controlled environment free from experimental noise, enabling robust benchmarking of analytical pipelines, optimization of bioinformatics tools, and validation of novel computational methodologies. The dataset's architecture allows for systematic evaluation of sensitivity, specificity, and scalability across different analytical approaches, making it particularly valuable for methodological development in the incorporation of NAM data to our methodological pipelines. The use of synthetic datasets allows us to rigorously evaluate the performance of our approach under controlled conditions, identify potential limitations, and refine the methodology before its application to real omics data from the OBERON project. This step is of significant importance for establishing confidence in the accuracy and reproducibility of our results.

The present workflow of our approach is showcased in Figure 20. This figure outlines the integration of transcriptomics data with the INTEGRA platform and its connection to the PARC model network via an API interface. While the methodology is still under development, the goal is to enable comprehensive processing and

analysis of complex omics data, including potential gene-gene and gene-disease interactions in order to incorporate NAM data into the methodological pipeline. Future work aims to enrich these datasets with carcinogenic endpoints, with the objective of providing a deeper understanding of the health impacts associated with specific genetic mechanisms. Once fully implemented, this approach is expected to support toxicological and disease-related research by generating actionable insights. The envisioned output format of the current version of our approach is presented in Figure 21, designed to summarize key findings such as: (a) Gene-Gene Interactions: Identification of potential regulatory networks or pathways, and (b) Gene-Disease Interactions: Mapping of genetic factors to specific diseases to aid in biomarker discovery. This will be the basis for the development of AONs.

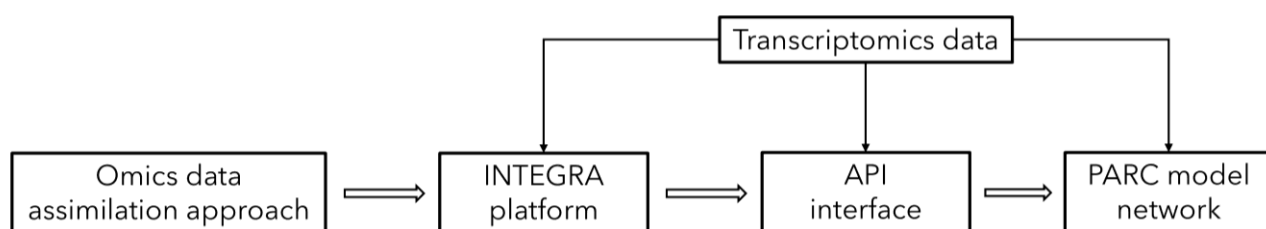


Figure 20. Interconnections of the developing pipeline with the PARC model network.

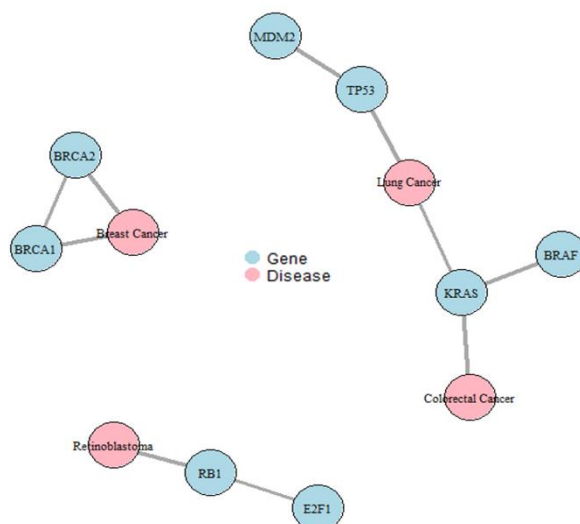


Figure 21. An instance of gene-gene and gene-disease interactions resulted from transcriptomic data enrichment and the application of our approach and the basis for the development of AONs. The dataset employed herein corresponds to the synthetic one that we have employed for development purposes.

3.7.3 Validation

The validation of our methodology will be conducted using *in vitro* transcriptomics data on bisphenols (BPA, BPS, BPF) generated within the framework of the OBERON project [56]. It is noteworthy that multiple omics datasets introduced under the OBERON project—such as metabolomics, transcriptomics, and multi-omics for multiple other chemical families—will be utilised in the subsequent steps of our approach. It is important to note that we have not yet worked with real data, as we are in the process of finalising data agreements with the data owners involved in the project. The OBERON project, funded under the European Union’s Horizon 2020 framework, was a cutting-edge initiative addressing the challenges posed by endocrine-disrupting chemicals (EDCs). EDCs are compounds that interfere with hormonal systems and may lead to serious health issues, including obesity, diabetes, and other metabolic disorders. Acknowledging the limitations of traditional testing methods, OBERON adopts innovative approaches to evaluate and mitigate the risks associated with these chemicals. The automated outputs of our approach will be tested on the outputs published by AUTH colleagues..

Additionally, the final validation step will include user experience and comments by transcriptomics experts of our group.

3.7.4 Next steps

The next steps of our approach involve integrating more human health endpoints into our computational pipeline. We aim to incorporate as many endpoints as possible to advance the development of AOPs into a robust computational framework for decision making. Furthermore, we plan to test and validate our strategy on additional omics datasets for other chemicals and extend their applicability to other omics domains, such as metabolomics and genomics. A key priority is the delivery of a well-defined API, which will be integrated into the methodological pipeline of the PARC model network to ensure widespread adoption and usability.

The developed methodology will be embedded into the PARC model network through an API interface and hosted under the INTEGRA platform, as depicted in Figure 15. This integration will enable seamless communication between our methodology and other models within the network, facilitating efficient data exchange and interoperability. The API will allow users to submit transcriptomics data, pre-processed according to our standardised template, for further analysis and endpoint prediction. It is critical that the developed solutions remain compatible with the broader PARC model network ecosystem, promoting collaborative development and enabling real-time application of the methodology across diverse use cases.

To ensure accessibility and ease of use, our analysis demonstration will feature straightforward steps and a user-friendly interface. We are developing a dedicated class, i.e., user form, that enables users to effortlessly connect their transcriptomics data to the relevant endpoints using a simple, standardised template. This tool is designed to streamline the integration of results and expand its functionality over time by incorporating additional endpoints as they become available. However, a significant challenge we have encountered during development is the lack of FAIR (Findable, Accessible, Interoperable, and Reusable) omics data templates. While initiatives like the OECD's Omics Reporting Framework (OORF) provide valuable reporting guidelines, they do not yet offer standardized computational templates (e.g., schemas for JSON/XML) [58]. The availability of such standardised templates would greatly simplify the integration of omics data into computational pipelines, enhancing the scalability, robustness, and overall impact of our methodology.

4 Alignment of workflows and network building

A combined bottom-up and top-down approach is used to develop the model network [1, 2]. The bottom-up approach builds specific workflows within (PARC) case studies, enhancing the functionality in modelling tools, and establishing connections between them as needed. Over time, it may address broader gaps. The top-down approach, guided by the conceptual framework (Figure 1) and technical standards, aims to enhance interoperability between modelling tools using a standardised approach. The approaches are closely integrated, iteratively shaping each other and the model network.

Based on the workflows presented in section 3.1 a network is emerging where modelling tools and workflows are linked at different levels:

- **Conceptual:** mapping workflows to the domains and modules in Figure 1 shows significant overlap, allowing workflows to integrate into broader assessments. For example, mixture RA (section 3.1) can incorporate chemical grouping (section 3.4), or an HBM-based mixture RA can be followed by exposure modelling (section 3.2) to identify the key exposure sources and routes for mixture risk drivers. Exposure models can also be validated using HBM data combining aspects of sections 3.1, 3.2 and 3.4. Workflows can also integrate NAM data (section 3.7). Similarly, EBD assessment (section **Erreur ! Source du renvoi introuvable.**) can utilise HBM data (3.1) or modelled exposures (3.2). **Efforts are underway to establish these links practically, with some workflows already implemented within the same interface** (3.1 - 3.4). A findable and searchable model inventory, accessible through e.g. <https://www.parc-models.eu/>, may also facilitate this process (see section 2.2). Further alignment of workflows for human and environmental RA is discussed in section 4.3.
- **Methodological:** linked to the conceptual overlap of workflows, workflows also share methodological overlap. For example, workflows for (cumulative) AEA (sections 3.1 and 3.2) build on EFSA's probabilistic

methodology for dietary cumulative RA [5, 16, 59]. While the extent of methodological alignment depends on context and application, **all workflows face the challenge of integrating multiple modelling steps and tools, requiring consistent propagation of outputs and uncertainties**. To address this, many workflows already use or plan to adopt MC frameworks, closely aligned with e.g. EFSA's uncertainty assessment guidance [5, 16, 60-62]. Additionally, the workflow in section 3.4 is linked to ongoing work on uncertainty analysis within PARC WP7, **contributing to a broader strategy for harmonised uncertainty assessment across the model network**. Strengthening these methodological connections will improve consistency, transparency and interoperability across workflows.

- **Technical:** building on the workflows described in section 0, a network of interconnected modelling tools is emerging. At the core of this network are key **hubs** – broad model platforms spanning multiple domains in Figure 1 that serve as primary access points for workflows. As mentioned in sections 2.2 and 0, MCRA, STOP and INTEGRA are the current hubs in the network, but other hubs may emerge over time. Connected to these hubs are nodes representing data sources and modelling tools. Different types of connections have been established within the workflows / network:
 - **Manual data exchange** between tools and data sources utilising harmonised data formats. Examples are the PARC harmonised HBM data format. Through this format, and the associated tools developed by VITO, individual-level HBM data is linked in several workflows (sections 3.1, **Erreur ! Source du renvoi introuvable.** and 3.4). Another example is the harmonised data format for exposure estimates described in section 3.2. It currently links consumer product exposure estimates obtained by PACEM to the wider AEA workflow in MCRA.
 - **Automatic data exchange:** in the PARC model network, we actively promote automated connections between tools as a way to improve interoperability, see AD8.4. However, full automation is not always feasible due to factors such as absence of common standards, technical constraints, or limited development capacity, making manual data exchange sometimes necessary. We have explored different approaches to automation, taking into account the specific characteristics of the tools involved, technical limitations and development possibilities.
 - **Embedding of tools** within a key hub to automate the execution of a modelling step by this tool. An example are exposure assessment models for different sources that are available in the modelling tool RSEXPO. As described in section 3.2, due to the design of RSEXPO (an RShiny app) automated linking to this tool was not feasible. Therefore, it is reimplemented in MCRA. Another example is a workflow for mixture selection which was described in a previous deliverable. Here a network analysis method [63], provided as an R script, was linked to MCRA by directly embedding it in the tool.
 - **Linking via APIs:** for standalone tools and tools embedded in software libraries (e.g., R packages) it is also possible to connect them via (language specific or generic) APIs within the model network. An example is the link between MCRA and the PROAST R package for dose response modelling (not discussed in this deliverable)²⁶.
 - **Pluggable models:** overlap between specific classes of models across workflows is observed, particularly for QSAR and PBK models, which are widely used. **These models serve as bridges in the emerging model network, connecting one or more key hubs.** Due to their structural similarities, a high level of harmonisation and standardisation can be achieved within each of these model classes, to enable straightforward implementation in workflows. For QSAR models, broad platforms such as VEGA already provide (partial) support for this. In contrast, PBK models currently lack interoperability, making their reuse across workflows challenging. **To address this, the development of a standardised framework for PBK model exchange is crucial** (see section 4.1). Models implemented in this standardised format should be directly pluggable in a workflow.

Linking via web APIs: for (larger) web-based modelling platforms, data exchange via web API connections provides another technical solution to exchange data between nodes of the model network. This offers a larger potential to automate a workflow while using the latest version of the modelling tool of interest. Within the model network, key nodes, such as the MCRA web platform and INTEGRA have been equipped with a web API. Ongoing efforts focus on further integrate the workflows into a broader model network. Section 4.1 outlines the development of a FAIR PBK modelling standard, and associated tooling, aiming to enhance their seamless incorporation into workflows. The next section discusses uncertainty analysis within model classes and workflows.

²⁶ <https://www.rivm.nl/en/proast>

At this stage, it provides a preliminary overview of key aspects to consider (mainly from the perspective of the workflow presented in section 3.4) rather than a fully developed strategy, serving as an initial step towards a structured approach. Finally, section 4.3 touches on the alignment of the workflows for human and environmental effects.

4.1 Use of FAIR PBK models in workflows

4.1.1 Description

PBK models play a vital role in various workflows for RA to derive internal exposures from external exposures and vice versa. It is recognised that there is no single PBK model that is suitable for all regulatory or RA purposes. Rather, a variety of PBK models are required to address the variety of questions for RA. For some cases, generic PBK models, applicable to a broad range of chemicals, may be used while other scenarios require specific and specialised models. Given this huge variety, it is essential to have a standardised (exchange) format for implementing PBK models, so that these can be easily integrated (or plugged) into various workflows for RA [64]. Such a standard does not yet exist, therefore, within the PARC model network, a FAIR PBK model standard is proposed to fill this gap.

Central to the proposed standard for FAIR PBK models is the model development and (re)use workflow depicted in Figure 22. In this workflow, both model developers and model users in principle develop and use these models within their own modelling/programming environments. The standard, however, provides a harmonised exchange format that enables mapping between different environments. The standard comprises a technical file format for describing PBK models and includes means to annotate model units of measurements (e.g., time resolution, parameter units) and a mechanism for annotating the model's applicability domain (e.g., the chemical class and species for which the model is suitable) and model elements (e.g., to identify blood compartments and parameters). The proposed FAIR PBK standard uses SBML as an exchange format [64-66], combined with an essential set of rules for specification of models units and semantic annotation of the model and model elements, using the PBPKO ontology currently developed in PARC WP7.

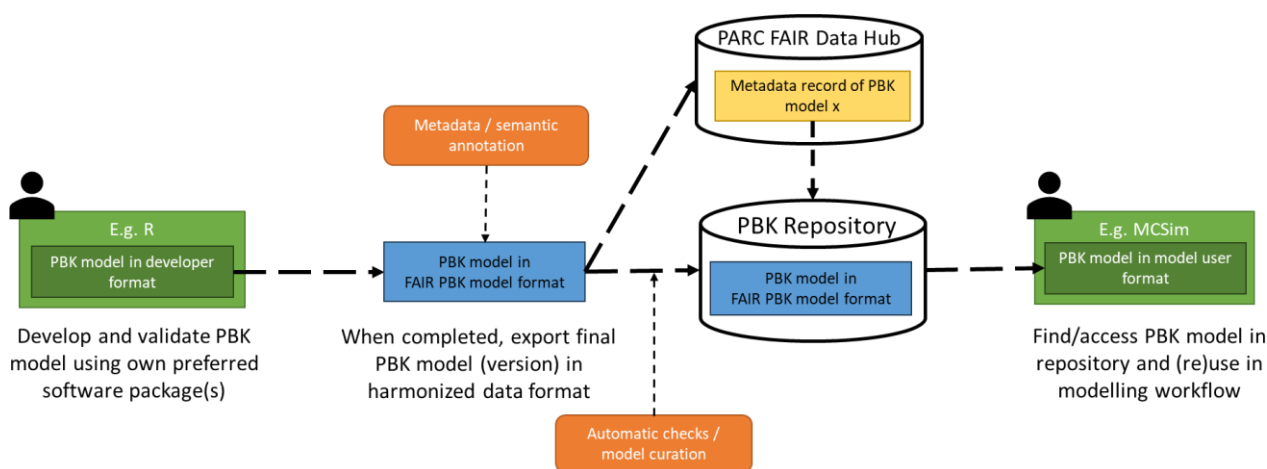


Figure 22 Diagram of the envisioned workflow of PBK model development and (re)use using a harmonised (FAIR) exchange format.

4.1.2 Demonstration

The use/benefit of the proposed FAIR PBK standard is demonstrated by implementation of a proof-of-principle of support for FAIR PBK models in the MCRA toolbox (see e.g. section 3.2) and implementation of a prototype FAIR PBK inspector tool to demonstrate and assist in PBK model annotation to comply to the standard. **Erreur ! Source du renvoi introuvable.** shows screenshots of the FAIR PBK inspector tool that demonstrates and assists in PBK model annotation to comply with the standard.

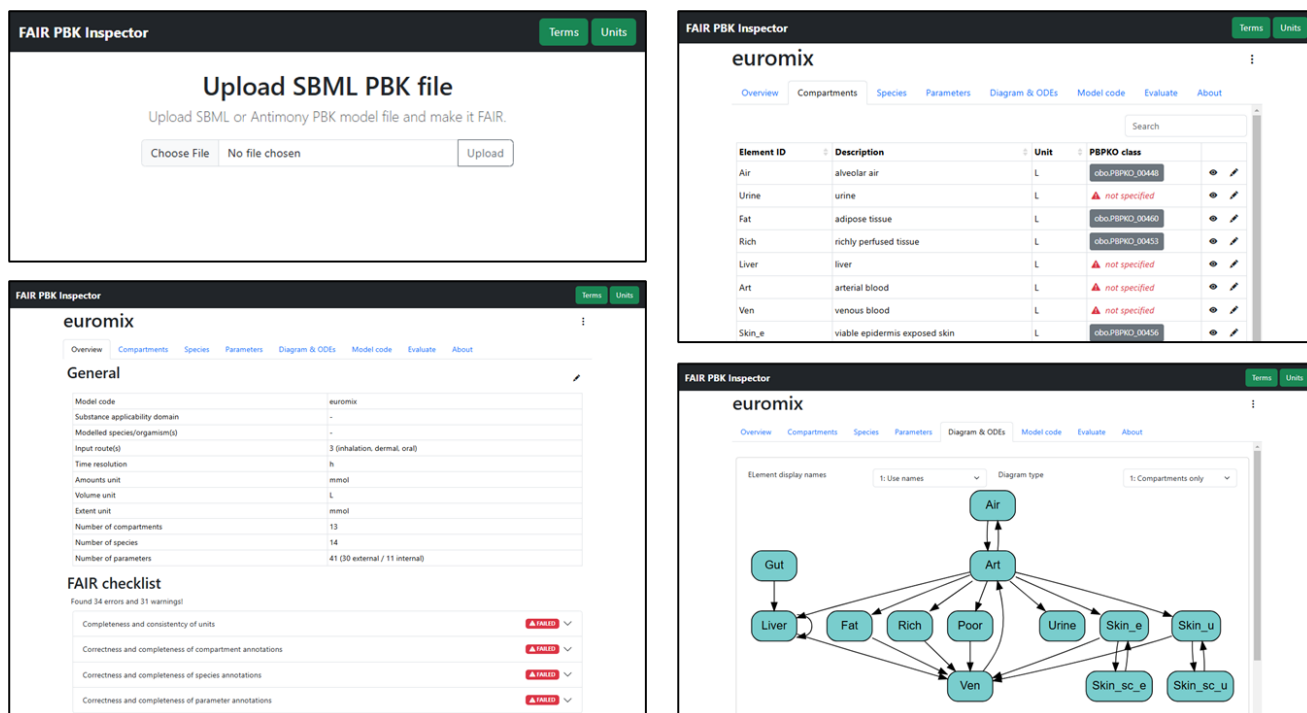


Figure 23 Screenshots of a demonstrative FAIR PBK inspector tool for assisting in annotation of SBML PBK models to make them FAIR. Top left: screen to upload an SBML file. Bottom left: overview of the uploaded PBK model and results of various FAIR checks. Top right: details on the compartments of the uploaded PBK models, including specification of units and annotation with PBPK terms. Bottom right: visual representation of the uploaded PBK model, demonstrating how model information is automatically extracted from this file format.

4.1.3 Uptake of the standard

For this FAIR PBK standard, community uptake and uptake of support in modelling tools is essential. Increased availability of FAIR PBK models creates awareness and an incentive for tool developers to implement support for the standard in modelling tools and, vice versa, increased tool support creates an incentive for PBK modelers to develop models according to the standard. Initiating such a feedback loop is essential for the success of this standard, which is done by building up an initial collection of FAIR PBK models, implementation of tooling (e.g., the FAIR PBK inspector) and tool support (e.g., support for FAIR PBK models in MCRA), and demonstrating their use in workflows (e.g., the workflow on aggregate exposure assessment). Within PARC task 6.2.2 this process has been initiated and several models are being (re-)implemented according to the FAIR PBK standard. The model code of the EuroMix PBK model re-implementation according to the standard is publicly available²⁷.

4.1.4 Next steps

At present, the FAIR PBK model standard is still in an early stage of development. Initial papers defining the framework and presenting the FAIR PBK inspector are in preparation. It is being developed simultaneously with the PBKO ontology, which plays a key role in developing this standard. As previously noted, community adoption—initially within PARC and eventually beyond—is considered crucial, along with continued alignment with international guidelines, such as the OECD guidelines [3]. The development of this standard follows a stagewise approach, iteratively developing more convincing examples and involving more people in the development.

The next step in the development is to work towards release of a first complete alpha version of the standard and the ontology that may be used for demonstrating, testing and validation by converting and annotating existing PBK models, relevant in PARC to this standard. On the PBK model development side, this will be done in collaboration with PARC task 6.2.2, and particularly focusing on implementation of PBK models needed for the workflows of PARC task 6.2.1. On the side of PBK model consumers (and tools), it involves implementation of support of this standard. One of the next steps is to further develop the current proof-of-principle implementation of support for FAIR (SBML) PBK models in the MCRA toolbox.

²⁷ <https://github.com/rivm-syso/euromix-to-sbml>

4.2 Towards a strategy for uncertainty analysis in workflows

A review of uncertainty methods conducted in Y3 has revealed that terms such as uncertainty, variability, heterogeneity, and sometimes also sensitivity, are used by different authors with slightly varying meanings. In the context of chemical, environmental, biomonitoring, and, more generally, mathematical modelling, variability is most commonly understood as an inherent characteristic of the studied population. While additional parameters may help explain some of this variability in the population, such explanations are often not useful or efficient from the perspective of the examined quantity. For example, variability can arise from factors such as varying levels of precipitation within a study area, natural differences in consumption patterns among individuals in a dietary study, or differences in functional groups within the molecular structure of substances whose toxicity is assessed using QSAR models. Knowing these circumstances might help to explain part of the variability in the population. On the other hand, uncertainty is an undesirable characteristic, representing a lack of certainty regarding either the model inputs (including inaccuracies in parameter measurements), the model structure and parametrisation (such as simplifications of the modelled system or approximations in the solution of the model), and consequently the model outputs. Uncertainty may be understood as a residual variability. It is not addressed by known parameters; however, it should be quantified whenever possible and considered when interpreting model results or using model outputs as inputs in another model within a model network. Examples include inaccuracies in rainfall measurements due to the use of different instruments, insufficient detail in food consumption questionnaires, or inadequate training datasets for a classification task in a QSAR model. Uncertainty is associated with the estimated quantities for each individual, reflecting variability in measurements, model inputs, and personal exposure estimates rather than a fixed value applied to the entire population.

Both, variability and uncertainty can be represented using similar mathematical frameworks, with the most common approach for quantitative assessment being the statistical probability distribution.

Sensitivity can be defined as the impact of potential changes (or errors) in input data on predicted model outputs and system performance indices. It is often conceptualised as the ratio between the variability/uncertainty in model outputs and their corresponding inputs. Sensitivity analysis can be used to investigate the propagation of both variability and uncertainty in a similar manner, even without distinguishing between them. It serves as a tool for (i) prioritising sources of uncertainty and identifying opportunities for minimising uncertainty, and (ii) investigating the propagation of uncertainty within models.

There are two principal approaches to addressing model uncertainty: qualitative and quantitative assessment. While the qualitative assessment can't be omitted from any of the modelling domains, it becomes challenging to incorporate when integrating models into more complex networks. In contrast, quantitative assessment allows for the use of precise mathematical frameworks in sensitivity analysis, enabling both the identification (and potential subsequent optimisation) of key sources of uncertainty, as well as the characterisation of uncertainty propagation from one model to another.

Typical qualitative measures of uncertainty rely on expert judgment, ranging from informal discussions and estimates to structured expert knowledge elicitation processes involving panels of multiple experts. They may cover different types of uncertainty, such as:

- Assessment of the scenario uncertainty (adequacy of selected model and its structure, long-term stability of the model inputs and/or parametrisation, outer conditions, etc.).
- Awareness about limitations due to biased inputs (selection bias, uncovered dependencies between inputs, nonrepresentative samples, etc.). With a good knowledge of model inputs and parameters, these limitations may be sometimes quantified and treated by quantitative approaches.
- Limited range of inputs for which the model may be used (extrapolation uncertainty).
- Discussion on applicability of the model results and the interpretation uncertainty when relating results of model simulations to reality.

The quantitative approaches encompass:

- Interval arithmetic, usually confidence intervals of model inputs/parameters and outputs in form of multiples of the standard deviation or quantiles. This is a minimalistic version of the probabilistic distribution approach in cases where well defined statistical distributions are expected.

- Probabilistic distribution using parametric or empirical stochastic distributions, either continuous (in case of numeric regression or continuous models) or discrete (probability of belonging to classes or clusters).
- Nonstochastic numerical expressions (e.g. posterior inclusion probabilities).
- Reliability categories.
- Fuzzy sets and other approaches (such as rough sets theory) which are rather rare.

Given assumptions about the probabilistic nature of input values (quantities), theoretical parametric distributions such as log-normal, normal, binomial, uniform, or Tweedie distributions may be employed. The main advantage of parametric distributions lies in their ability to be described by a small number of parameters (typically 2 or 3), which enables relatively simple operations, such as summation or multiplication, on these distributions. This can be fully parametric (e.g., summing normal distributions yields a normal distribution) or involve approximations (e.g., the Fenton-Wilkinson approximation for summing log-normal distributions). However, these advantages are less relevant in numerically solved models, where inputs are generally not defined in terms of statistical distributions, and the model must be run repeatedly using randomly generated values. The main disadvantages of theoretical distributions lie in their inability to fully represent all real-world empirical distributions and the approximate nature of some operations.

In contrast, empirical distributions preserve the true shape of the measured input data, either with high accuracy (by retaining every individual measurement) or in a simplified manner (through histogram-like discretisation or smoothing). This approach provides a direct method for characterising the probabilistic nature of inputs but can only be handled using MC simulations and derived bootstrapping techniques. When dealing with large datasets and/or complex models, using empirical distributions may become computationally intensive and slow. Our ongoing research aims to implement both empirical and parametric distributions with approximate operations and compare their results to recommend optimal usage strategies for workflows in the integrative model network.

Practical experience suggests that external concentrations (e.g., exposure to chemicals) typically follow log-normal distributions, while internal concentrations (such as body levels or variables within PBK models) may follow a broader range of distributions, including the normal distribution. A specific case involves food questionnaires, which often exhibit strongly zero-inflated distributions (e.g., “I never eat peanuts”) that can be effectively modelled using the less common Tweedie distribution or zero-inflated models (e.g. a betabinomial-normal model). Multiplying Tweedie-distributed data by log-normally distributed concentrations and summing them together can be achieved using approximations developed in our case study, “*Uncertainty in PFAS Exposure,*” as discussed above.

4.2.1 Methods for local and global sensitivity assessment

Two methods are commonly used to assess the sensitivity of model inputs. While local sensitivity analysis focuses on individual model inputs or parameters by varying them around their typical or reference values and examining their local impact on the model output through linear approximations, global sensitivity analysis takes a more comprehensive approach, investigating the uncertainty of factors across the entire feasible space of the model parameters. Local sensitivity analysis, therefore, explores how small changes in model parameters/inputs affect the model performance. It is popular due to its simplicity and low computational cost; however, it has significant limitations. For nonlinear models, the results of local sensitivity analysis can be biased due to the assumptions of independence and limited exploration of the model inputs. If interactions between model inputs exist, local sensitivity analysis may underestimate their importance, as it does not account for such interactions. As local sensitivity analysis only partially and locally explores the parametric space of the model, it should not be considered a suitable approach for nonlinear models. In contrast, global sensitivity analysis examines the effects of each parameter on the model output across the full range of its meaningful values, including potential interactions with other parameters. For nonlinear models, global sensitivity analysis is generally preferred, except for preliminary investigations of the importance of parameters.

The following methods for sensitivity assessment were identified and described during our work on the *Overview of Methods for Uncertainty Assessment in Physiologically Based Kinetic Models*.

4.2.2 Methods for sensitivity analysis

Analytical solution using parametric distributions: Estimating sensitivity coefficients as derivatives of a model output variable with respect to an input variable or parameter. A number of sensitivity analysis methods use these coefficients. First-order and approximate first-order sensitivity analyses are two such methods.

Method of finite differences: Simple and straightforward numeric method replacing derivatives (which are not known) by their estimates based on finite differences. Does not need the analytically expressed solution of the model.

Variance-based method: the method utilises the values of partial derivatives or their estimates of the model output in respect to its inputs. This means the method has all disadvantages of the abovementioned methods. Moreover, it is only applicable for parametric inputs for which the variance may be well specified.

Sobol' method: the variance-based method which decomposes the variance of the output into fractions which can be attributed to inputs and their interactions. Sobol' method decomposes the total variance into orthogonal contributions of the inputs and their combinations.

Simple Monte Carlo Sampling: Simple method generating random combinations of the input parameters (all-at-a-time) using large number of sets of parameters. However, this method is extremely slow for models with multiple parameters/inputs.

Latin Hypercube Sampling (LHS): Significantly reduces the time for testing parameter combinations from the whole multidimensional parameter space compared to simple MC method. Disadvantages The time efficiency is better but for more complex models with multiple parameters may be still quite high.

Generalised Likelihood (Uncertainty) Estimation (GLUE): prevents over-parameterisation (ie. strong interaction between model parameters) by estimating posterior joint probability distribution functions of the input parameters. May be also used for model calibration (as follows).

Fourier Amplitude Sensitivity Testing (FAST): one of the most popular uncertainty and sensitivity analysis techniques. It uses a periodic sampling approach and a Fourier transformation to decompose the variance of a model output into partial orthogonal variances contributed by different model inputs.

Extended Fourier Amplitude Sensitivity Testing (eFAST): based on the FAST method but with increased efficiency. It consists of a definition of new sets of parametric equations for a search-curve exploring an input space, a selection of frequencies for the parametric equations, and a procedure to estimate their contributions to the total variance.

Multi-test Extended Fourier Amplitude Sensitivity Testing (MeFAST): Another extension of eFAST ensures critical conditions must be met for an unbiased sampling scheme. MeFAST tests convergence measures and convergence plots to assess the robustness of the sensitivity indices and to assess whether more simulations are needed.

Morris's method: utilises consequent changes of model inputs (one-at-a-time) to facilitate a global sensitivity analysis by mapping changes in model outputs. The method deals efficiently with models containing hundreds of input factors without relying on strict assumptions about the model, such as additivity or monotonicity.

Following our experience, we suggest eFAST or Morris's method as the most suitable for implementation into the integrative modelling network. Additionally, in certain contexts, such as probabilistic dietary mixture RA following EFSA methodology, an established practice for uncertainty assessment already exists and should also be supported.

4.2.3 Methods for calibrating mathematical models

Another group of methods serve for a calibration of PBK model parameters with known inputs and output of the model. During our work on the Overview of Methods for the Uncertainty Assessment in PBK Models we have collected and briefly described the following methods:

Hamiltonian MCMC (without random walk) [67], Bayesian Metropolis-Hastings (MHMCMC)²⁸, Bayesian delayed rejection adaptive Metropolis-Hastings (DRAM) [68], Bayesian Gibbs sampler [69], Bayesian reversible jump MCMC

²⁸ <https://www.r-Bloggers.Com/2019/04/Understanding-Bayesian-Inference-with-a-Simple-Example-in-r/>

[70], *Quasi-random parametric expectation maximisation (QRPEM)*²⁹, *Nonparametric adaptive grid estimation (NPAG)* [71].is

Following our experience, we suggest the Bayesian Metropolis-Hastings (MHMCMC) method, possibly with some additional optimisation of the random walk steps as the most suitable for implementation into the integrative modelling network.

4.2.4 Uncertainty methods/strategies by model domains

In the following paragraphs, specifics of different chemical/environmental/biological model domains are briefly described, showing differences between them and applicability of the methods described above.

PBK modelling

Main focus in the field of PBK models is put on model parameterisation, however the variability/uncertainty and modelling errors are also of interest [3]. Since typical PBK models are numerically solved sets of differential equations, either in deterministic or probabilistic form, the absolute majority of the reviewed uncertainty assessment focuses on methods of local and especially global sensitivity as discussed above. This also means that all the methods of uncertainty/sensitivity assessment for PBK models are based on repeated runs of the models with variable inputs based on bootstrapping or MC simulations. An analytical solution is not possible.

Environmental modelling

The situation for the most common compartmental environmental models is similar to that of PBK models. Since these models are composed of complex sets of differential equations, analytical solutions are generally not feasible, making parametric distributions less advantageous. Instead, empirical distributions and global sensitivity methods are recommended for uncertainty analysis.

QSAR modelling

The main sources of uncertainty within the QSAR modelling are often classified into four groups : (i) the uncertainty of chemical (structural descriptors such as measured or calculated physico-chemical properties and a meaningful selection of descriptors used as the model inputs); (ii) the uncertainty in the biological action (input data uncertainty, endpoint relevance and a knowledge on the mechanism of the biological action); (iii) mathematical/statistical uncertainty (performance and reproducibility/accuracy of the model), and (iv) the uncertainty in the model applicability (relevance and the domain of the model application).

QSAR models are reliable within their applicability domain (AD) but have limitations when predicting the properties of compounds in regions of chemical space that are not well represented by the training set. The AD indicates the region of chemical space where the model can confidently predict properties or activities of new compounds. QSAR models are most reliable for compounds structurally similar to those in the training set. The prediction error increases as the distance between a new compound and the nearest element in the training set grows. This can be measured by e.g. the Tanimoto distance using fingerprints, which is a method of quantifying structural similarity between compounds. A common approach to defining the AD involves setting a threshold (e.g., 0.4 or 0.6) for the Tanimoto distance from the nearest training set compound. For QSAR models classifying into categories (e.g. toxic active/inactive compound), the probability of a compound being classified into a given category can be used to determine the reliability of the model. In practice, this means that in addition to the prediction of the compound property, we should also extract from the QSAR model information on whether the result is within AD, the likelihood of the model estimate (e.g., the explained variability of the QSAR model, classification accuracy and precision) to determine the uncertainty of the estimate. It is possible to also combine the results of available QSAR models, built on different training sets, to predict a given property.

An alternative to AD that has seen uptake in QSAR is conformal prediction, a statistical framework used to quantify uncertainty in AI modelling, providing valid and reliable confidence estimates for predictions. Unlike traditional methods that generate single-point predictions, conformal prediction produces prediction intervals or sets, ensuring a predefined confidence level. By leveraging past model errors and applying rigorous statistical principles, it offers a robust approach to estimating uncertainty for predictions. This methodology enhances decision-making by indicating the reliability of individual predictions, offering a compelling alternative or complement to traditional AD.

²⁹ https://www.page-meeting.org/pdf_assets/5496-page2012posterleary.pdf

Exposure modelling

Exposure models are mostly presented by deterministic models using specific input values and producing a single output for a given set of inputs following a specific, fixed mathematical relationship or formula. For this reason, exposure models are often considered as models with no representation of uncertainty. In this case, the main source of uncertainty is represented by the input values (e.g. estimation precision) rather than by the model itself, and uncertainty will propagate directly from the input to the outcome. Thus, the measurement error will contribute the most to the outcome uncertainty neglecting the model uncertainty. Moreover, predictions of deterministic models are often required as input to probabilistic models and a single-value output isn't appropriate in this scenario.

There are several approaches to dealing with uncertainty estimation related to the outcomes of such models [72].

- Expert assessment. Experts' opinions in the field could help to estimate the variance of input and output values, and relationships in scenarios where direct observations are impractical or expensive. Thus, precision estimation or variability based on the expert assessment could be useful for assessing uncertainty around both the input and output.
- Model sensitivity analysis. This type of analysis is described above in detail. Combined with the expert assessment, sensitivity analysis provides better uncertainty estimation of the output. However, this method could be computationally demanding and could be substituted with the method below.
- Model emulation. It's used for complex exposure models where a simplified statistical approximation is used instead of the original complex model. Gaussian processes are commonly applied to build emulators, providing a probabilistic distribution for model outputs.
- Temporal variability in deterministic models. In case of exposure models' temporal variability refers to changes in the model output over time. Thus, the range of changes observed over time is treated as an uncertainty estimate of the model prediction (can be used as a proxy of the model uncertainty). However, this approach is likely to overestimate or underestimate the true uncertainty.
- Multiple models. Several models (different structures, parametrisation etc.) can be used to estimate the same outcome. Thus, using several models provides different estimates of the targeted outcome and can help evaluate predictive uncertainty. Additionally, models can be weighed using techniques like Bayesian model averaging or expert opinion.
- Data-based approaches. This approach includes utilising data from similar cases or testing model performance using methods like cross-validation and bootstrapping to explore variability.

In contrast, probabilistic exposure models, such as for dietary exposure presented in section 3.2 employ a MC framework to quantify both variability and uncertainty in the exposure estimates.

Dose response modelling

In Y3 we have not started with investigating specifics of uncertainty assessment within the toxicological models. Comparison of different concepts and probabilistic distributions will be investigated in Y4.

Morphological modelling

During Y3, work was initiated on uncertainty modelling for morphological modelling based on Cell Painting data and conformal prediction methodology. Chemical grouping by morphological profiling was also initiated based on prediction intervals from conformal prediction. Both uncertainty for classification and regression settings was investigated and this work will continue during Y4.

4.3 Aligning the workflows for human health and environmental effects

To enhance consistency and interoperability across human health and environmental RA models, efforts will focus on identifying areas of improved alignment between human and environmental models. This alignment will support standardised processing of human health effects within the PARC model network while ensuring integration with environmental exposure assessment frameworks, where this can be achieved. The harmonisation process will be guided by FAIR principles, leveraging controlled vocabularies, ontologies, and terminologies to improve data interoperability and reuse of common models, when feasible.

As part of this effort, the alignment between MCRA, STOP, VEGA-hub and AOP network modelling is envisioned. MCRA's well-established methodologies in chemical RA provide a foundation for ensuring that data formats between systems are compatible. Where direct alignment with MCRA's existing formats is feasible, adjustments to

STOP will be made accordingly; where discrepancies exist, automated mapping tools can be developed to facilitate seamless data translation within the PARC data hub.

In parallel, efforts to align ongoing environmental AEP development with PARC's human AEA models (e.g., AD6.3, WP6) will be prioritised. Given that AEP development is still in its early stages, this alignment provides an opportunity to harmonise methodologies across domains from the outset. Close collaboration between human and environmental modelling communities will ensure that exposure assessment workflows are consistent, drawing upon shared research and expertise to strengthen RA capabilities.

By fostering greater integration between MCRA, STOP, other modelling tools, this alignment initiative will promote a more comprehensive and standardised approach to chemical RA, bridging human health and environmental perspectives within the broader PARC model network.

5 Conclusion

The PARC model network aims to support harmonised and scalable IRAs in both scientific and regulatory contexts. Within this network, multiple workflows are being developed. These include aggregate (cumulative) RAs based on either modelled exposures or measured exposures from HBM data, estimates of the attributable burden of disease using the same exposure inputs, and the integration of NAMs. While most workflows focus on human populations, the network will also include a dedicated workflow for environmental assessments. The applicability of these workflows has been demonstrated in this deliverable.

An updated overarching framework illustrates how these workflows contribute to a broader network for IRA at the conceptual level. The workflow development also plays a key role in shaping the actual model network in a bottom-up fashion. A structured network is emerging in which broad modelling platforms - particularly MCRA, INTEGRA and STOP - serve as key hubs, linking various other modelling tools and data sources. These hubs provide UIs for workflows. Additionally, specific model classes, such as PBK models, are frequently reused across workflows, further strengthening the connections between these key hubs.

The model network is being developed iteratively, with continuous refinement. In the next period, the overarching framework will be updated both conceptually, as needed, and technically, with a focus on establishing interoperability standards to support further refinement of the workflows. Conceptual updates include refining domains and modules, aligning, with e.g. ontology development or other activities in PARC WP7, and refining the framework for uncertainty assessment within workflows using insights gained from the case study comparing modelled exposures with HBM concentrations.

To enhance the findability and accessibility of modelling tools and workflows, efforts will be made to linking the model inventory with the PARC model network website (<https://www.parc-models.eu/>) and the PARC data hub.

Finally, a key technical priority in Y4 is to advance FAIR PBK framework, to support aggregate exposure modelling (Section 3.2). The framework will be refined with the aim of aligning with OECD guidance and will be used in the PBK models developed in PARC T6.2 to generate (open source) FAIR PBK models for the substances considered in the T6.2 aggregate exposure modelling case studies. Early (development) versions are available for the EuroMix PBK model³⁰ and a PFAS PBK model³¹.

For the workflows, short and long-term next steps were outlined in the relevant subsections of section 0. These updates will be guided by the updated overarching framework. A key focus will be further development and application of workflows in co-creation with partners such as T6.2 (workflows in sections 3.1 - 3.4). In addition, as noted in section 4, strengthening the link between workflows, such as measured exposures (HBM data) and modelled exposures (touching upon sections 3.1 - 3.3, and 4.1) through the use of FAIR PBK models, will be pursued. As workflows evolve, proper versioning of both workflows, tools, and data formats will be critical to ensure transparency and reproducibility. This aspect will therefore be taken into account, starting with the workflow for aggregate exposure assessment.

³⁰ <https://github.com/rivm-syso/euromix-to-sbml>

³¹ <https://github.com/rivm-syso/pfasPBK>

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