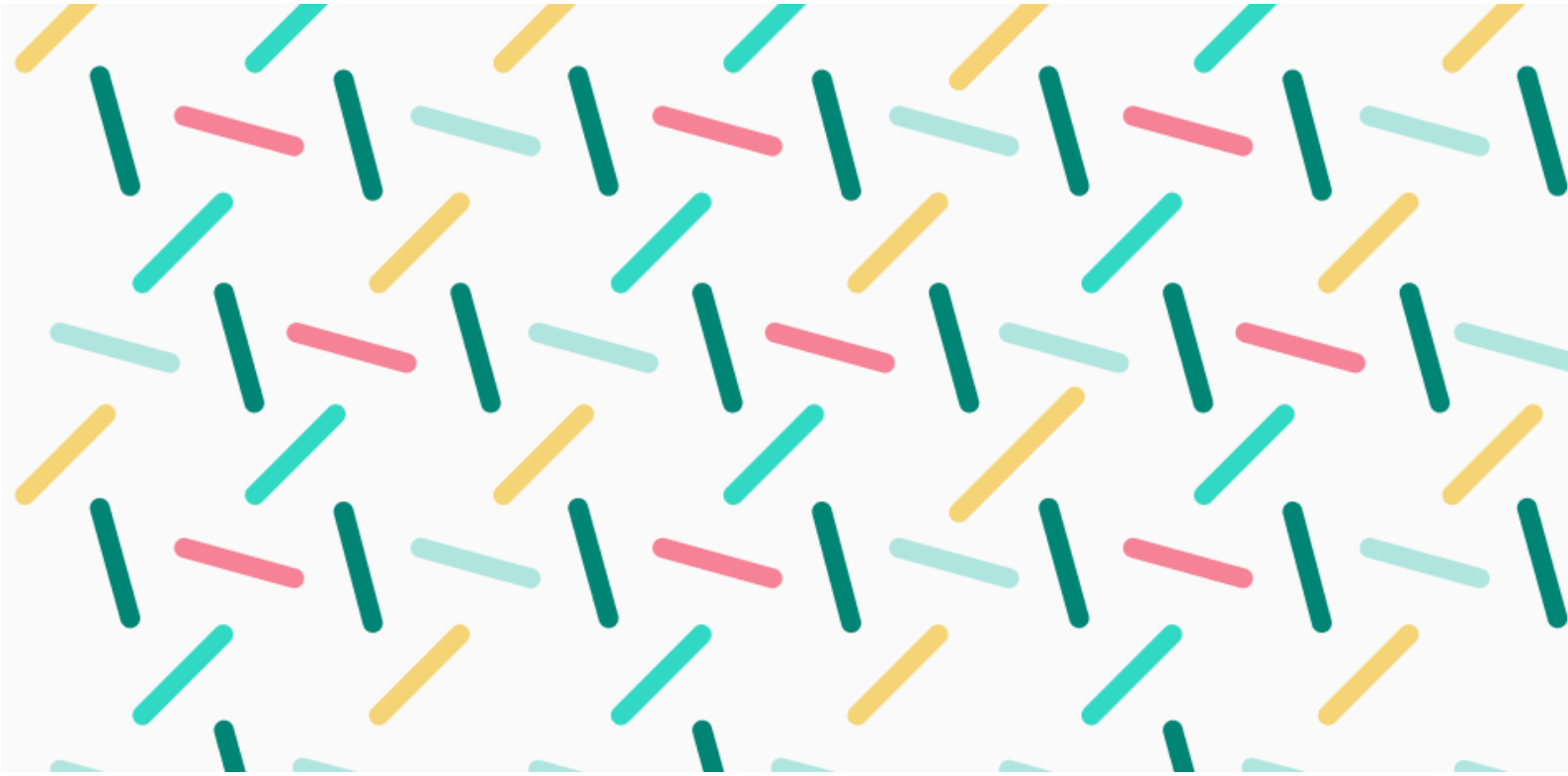
# Partnership for the Assessment of Risks from Chemicals

Additional Deliverable AD4.6

Concept paper regarding common definitions and short to medium term support to policy objectives of innovative (self-) sampling, suspect screening and NTS/EDA approaches

WP 4 – T4.3



PARC Partnership for the Assessment of Risks from Chemicals

Co-funded by the European Union

This partnership has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014.

| Technical reference | |
|---|---|
| Work package | WP 4 - Monitoring and Exposure |
| Task | T 4.3 – Innovative methods and tools for monitoring and surveys |
| Dissemination level[1] | PU |
| Lead Beneficiary/ Responsible AE | INRAE (FR), VUA (NL) |
| Contributing Participants | Participants: MU (CZ), ANSES (FR), BRGM (FR), EAWAG (CH), EHESP (FR), INRS (FR), JSI (SI), LNS (LU), MUI (AT), UAntwerpen (BE), UBA (DE), UFZ (DE), VITO (BE), WR (NL), EV-ILVO (BE), KWR (NL), JWGU (DE), KU Leuven (BE), CSIC (ES), ETH (CH), UL (SI) |
| Responsible author(s) | Elliott J. Price / MU / elliott.price@recetox.muni.cz |
| Co-authors | N/A |
| Reviewers internal to WP | Nicole Bandow / UBA (DE) / nicole.bandow@uba.de |
| Reviewers external to WP | Karl Lilja / Naturvårdsverket (SE) / Karl.Lilja@naturvardsverket.se<br>Anja Duffek / UBA (DE) / anja.duffek@uba.de |
| Due date of deliverable | 30.04.2023 |
| Actual submission date | 31.08.2023 |

[1] PU = Public

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

RE = Restricted to a group specified by the consortium (including the Commission Services)

CO = Confidential, only for members of the consortium (including the Commission Services)

## Document history

| Version | Date | Reviewer name/Institutions | Short description of changes |
|---|---|---|---|
| 1.0.0 | 25/07/2023 | Elliott J Price / MU / elliott.price@recetox.muni.cz | Changes based on reviewer comments external to WP. |
| 2.0.0 | 01/08/2023 | Elliott J Price / MU / elliott.price@recetox.muni.cz | Changes based on PARC CT comments |
| 3.0.0 | 18/08/2023 | Elliott J Price / MU / elliott.price@recetox.muni.cz | Changes based on PARC CT comments |

"Funded by the European Union. Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them."

## Table of contents

## Title, authors, affiliations, contact details

**Innovative methodologies for characterising human chemical exposure with a view to next-generation risk assessment**

Žiga Tkalec[1,2], Jean-Philippe Antignac[3], Nicole Bandow[4], Frederic M. Béen[5,6], Lidia Belova[7], Jos Bessems[8], Bruno Le Bizec[3], Werner Brack[9,10], German Cano-Sancho[3], Jade Chaker[11], Adrian Covaci[7], Nicolas Creusot[12], Arthur David[11], Laurent Debrauwer[13], Gaud Dervilly[3], Radu - Corneliu Duca[14,15], Valérie Fessard[16], Joan O. Grimalt[17], Thierry Guerin[18], Baninia Habchi[19], Helge Hecht[1], Juliane Hollender[20,21], Emilien L. Jamin[13], Jana Klánová[1], Tina Kosjek[2], Martin Krauss[22], Marja Lamoree[5], Gwenaelle Lavison-Bompard[23], Jeroen Meijer[5], Ruth Moeller[24], Hans Mol[25], Sophie Mompelat[16], An Van Nieuwenhuyse[15,26], Herbert Oberacher[27], Julien Parinet[23], Christof Van Poucke[28], Robert Roškar[29], Anne Togola[30], Jurij Trontelj[29] and Elliott J. Price[1,*]

[1] RECETOX, Faculty of Science, Masaryk University, Kotlarska 2, Brno, Czech Republic

[2] Jožef Stefan Institute, Department of Environmental Sciences, Ljubljana, Slovenia

[3] Oniris, INRAE, LABERCA, 44300 Nantes, France

[4] German Environment Agency, Laboratory for Water Analysis, Colditzstraße 34 12099 Berlin, Germany

[5] Vrije Universiteit Amsterdam, Amsterdam Institute for Life and Environment (A-LIFE), Section Chemistry for Environment and Health, De Boelelaan 1085, 1081 HV Amsterdam, the Netherlands

[6] KWR Water Research Institute, Nieuwegein, the Netherlands

[7] Toxicological Center, University of Antwerp, 2610 Wilrijk, Belgium

[8] Flemish Institute for Technological Research (VITO), Mol, Belgium

[9] Helmholtz Centre for Environmental Research GmbH – UFZ, Department of Effect-Directed Analysis, Permoserstraße 15, 04318 Leipzig, Germany

[10] Goethe University Frankfurt, Department of Evolutionary Ecology and Environmental Toxicology, Max-von-Laue-Strasse 13, 60438 Frankfurt, Germany

[11] Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, Rennes, France

[12] INRAE, French national research institute for agriculture, food & environment, UR1454 EABX, Bordeaux Metabolome, MetaboHub, Gazinet Cestas, France.

[13] Toxalim (Research Centre in Food Toxicology), INRAE UMR 1331, ENVT, INP-Purpan, Paul Sabatier University (UPS), Toulouse, France

[14] Unit Environmental Hygiene and Human Biological Monitoring, Department of Health Protection, Laboratoire National de Santé (LNS), 1 Rue Louis Rech, L-3555 Dudelange, Luxembourg

[15] Environment and Health, Department of Public Health and Primary Care, Katholieke Universiteit of Leuven (KU Leuven), 3000 Leuven, Belgium

[16] ANSES, French Agency for Food, Environmental and Occupational Health & Safety, Laboratory of Fougères, Toxicology of Contaminants Unit, 35306 Fougères, France

[17] Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Catalonia, Spain

[18] ANSES, French Agency for Food, Environmental and Occupational Health & Safety, Strategy and Programs Department, F-94701 Maisons-Alfort, France

[19] INRS, Département Toxicologie et Biométrologie Laboratoire Biométrologie 1, rue du Morvan - CS 60027 - 54519 Vandoeuvre Cedex

[20] Swiss Federal Institute of Aquatic Science and Technology – Eawag, 8600 Dübendorf, Switzerland

[21] Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, 8092 Zürich, Switzerland

[22] Helmholtz Centre for Environmental Research GmbH – UFZ, Department of Effect-Directed Analysis, Permoserstraße 15, 04318 Leipzig, Germany

[23] ANSES, French Agency for Food, Environmental and Occupational Health & Safety, Laboratory for Food Safety, Pesticides and Marine Biotoxins Unit, F-94701 Maisons-Alfort, France

[24] Unit Medical Expertise and Data Intelligence, Department of Health Protection, Laboratoire National de Santé (LNS), 1 Rue Louis Rech, L-3555 Dudelange, Luxembourg

[25] Wageningen Food Safety Research – part of Wageningen University and Research, Akkermaalsbos 2, 6708 WB, Wageningen, the Netherlands

[26] Department of Health Protection, Laboratoire National de Santé (LNS), 1 Rue Louis Rech, L-3555 Dudelange, Luxembourg

[27] Institute of Legal Medicine and Core Facility Metabolomics, Medical University of Insbruck, 6020 Innsbruck, Austria

[28] Flanders research institute for agriculture, fisheries and food (ILVO), Brusselsesteenweg 370, 9090 Melle, Belgium

[29] University of Ljubljana, Faculty of Pharmacy, Slovenia

[30] BRGM, 3 avenue Claude Guillemin, 45060 Orléans, France

Correspondence: Elliott J. Price; elliott.price@recetox.muni.cz; RECETOX, Faculty of Science, Masaryk University, Office: bldg. D29/209, Kamenice 753/5, 625 00 Brno, (ORCiD: (0000-0001-5691-7000)

Contact:

Žiga Tkalec, ziga.tkalec@recetox.muni.cz, (ORCiD: 0000-0003-4518-3923)

Jean-Philippe Antignac, jean-philippe.antignac@oniris-nantes.fr, (ORCID: 0000-0001-9512-9314)

Nicole Bandow, nicole.bandow@uba.de, (ORCiD: 0000-0003-4082-6125)

Frederic M. Béen, f.m.been@vu.nl, (ORCiD: 0000-0001-5910-3248)

Lidia Belova, lidia.belova@uantwerpen.be, (ORCiD: 0000-0001-7147-384X)

Jos Bessems, jos.bessems@vito.be, (ORCiD: 0000-0001-5718-0733)

Bruno Le Bizec, bruno.lebizec@oniris-nantes.fr (ORCiD: 0000-0002-0600-5895)

Werner Brack, werner.brack@ufz.de, (ORCiD: 0000-0001-9269-6524)

German Cano-Sancho, german.cano-sancho@oniris-nantes.fr, (ORCiD: 0000-0003-4111-4007)

Jade Chaker, jade.chaker@ehesp.fr, (ORCiD: 0000-0002-3680-4786)

Adrian Covaci, adrian.covaci@uantwerpen.be, (ORCiD: 0000-0003-0527-1136)

Nicolas Creusot, nicolas.creusot@inrae.fr, (ORCiD: 0000-0002-4240-1529)

Arthur David, arthur.david@ehesp.fr, (ORCiD: 0000-0001-8189-1826)

Laurent Debrauwer, laurent.debrauwer@inrae.fr, (ORCiD: 0000-0003-3600-0902)

Gaud Dervilly, gaud.dervilly@oniris-nantes.fr, (ORCiD: 0000-0002-1867-0008)

Radu Duca, radu.duca@lns.etat.lu, (ORCiD: 0000-0002-9350-3816)

Valérie Fessard, valerie.fessard@anses.fr (ORCiD: 0000-0001-9046-9117)

Joan O. Grimalt, joan.grimalt@idaea.csic.es, (ORCiD: 0000-0002-7391-5768)

Thierry Guerin, thierry.guerin@anses.fr, (ORCiD: 0000-0003-4060-6616)

Baninia Habchi, baninia.habchi@inrs.fr, (ORCiD: 0000-0001-9705-167X)

Helge Hecht, helge.hecht@recetox.muni.cz, (ORCiD: 0000-0001-6744-996X)

Juliane Hollender, juliane.hollender@eawag.ch, (ORCiD: 0000-0002-4660-274X)

Emilien L Jamin, emilien.jamin@inrae.fr, (ORCiD: 0000-0002-4568-9177)

Jana Klánová, jana.klanova@recetox.muni.cz, (ORCiD: 0000-0002-8818-5307)

Tina Kosjek, tina.kosjek@ijs.si, (ORCiD: 0000-0002-6421-0344)

Martin Krauss, martin.krauss@ufz.de (ORCiD: 0000-0002-0362-4244)

Marja Lamoree, marja.lamoree@vu.nl, (ORCiD: 0000-0002-7373-7738)

Gwenaelle Lavison-Bompard, gwenaelle.lavison-bompard@anses.fr (ORCiD: 0000-0001-6793-6290)

Jeroen Meijer, j.meijer@vu.nl, (ORCiD: 0000-0002-4551-6823)

Ruth Moeller, Ruth.Moeller@lns.etat.lu

Hans Mol, hans.mol@wur.nl, (ORCiD: 0000-0003-0087-6910)

Sophie Mompelat, sophie.mompelat@anses.fr, (ORCiD: 0000-0002-3063-1853)

An Van Nieuwenhuyse, An.vannieuwenhuyse@lns.etat.lu, (ORCiD: 0000-0003-3942-7012)

Herbert Oberacher, Herbert.oberacher@i-med.ac.at, (ORCiD: 0000-0002-0963-8268)

Julien Parinet, julien.parinet@anses.fr, (ORCiD: 0000-0003-0119-0929)

Christof Van Poucke, christof.vanpoucke@ilvo.vlaanderen.be, (ORCiD: 0000-0001-7566-925X)

Robert Roškar, robert.roskar@ffa.uni-lj.si, (ORCiD: 0000-0001-9863-9117)

Anne Togola, a.togola@brgm.fr, (ORCiD: 0000-0002-5393-1533)

Jurij Trontelj, jurij.trontej@ffa.uni-lj.si, (ORCiD: 0000-0002-2558-1425)

## Abstract

The chemical burden on the environment and human population is increasing. Consequently, regulatory risk assessment must keep pace to manage, reduce, and prevent adverse impacts on human and environmental health associated with hazardous chemicals. Surveillance of chemicals of known, emerging, or potential future concern, entering the environment-food-human continuum is needed to document the reality of risks posed by chemicals on ecosystem and human health from a one health perspective, feed into early warning systems and support public policies for exposure mitigation provisions and safe and sustainable by design strategies. The use of less-conventional sampling strategies and integration of full-scan, high-resolution mass spectrometry and effect-directed analysis in environmental and human monitoring programmes have the potential to enhance the screening and identification of a wider range of chemicals of known, emerging or potential future concern. Here, we outline the key needs and recommendations identified within the European Partnership for Assessment of Risks from Chemicals (PARC) project for leveraging these innovative methodologies to support the development of next-generation chemical risk assessment.

## Keywords

High-resolution mass spectrometry, effect-based methods, sampling strategies, chemical exposure, chemical risk assessment, effect-directed analysis

## Abbreviations

European Partnership for Assessment of Risks from Chemicals (PARC)

European Human Biomonitoring Initiative (HBM4EU)

human biomonitoring (HBM)

aggregated exposure pathway (AEP)

adverse outcome pathway (AOP)

HBM-based guidance values (HBM-GVs)

Mass spectrometry (MS)

gas chromatography (GC)

liquid chromatography (LC)

first MS (MS1)

selective ion monitoring (SIM)

multiple reaction monitoring (MRM)

parallel reaction monitoring (PRM)

limit of detection (LOD)

limit of quantification (LOQ)

tandem mass spectrometry (MS/MS)

second MS (MS2)

Full scan (Fs)

neutral loss (NL)

target-directed data dependent acquisition (tDDA)

neutral loss monitoring (NLM)

Fs data dependent acquistion (DDA)

Fs data independent acquisition (DIA)

Survey on Pesticide Mixtures in Europe (SPECIMEn)

Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances (NORMAN)

high-resolution mass spectrometry (HRMS)

Global Natural Products Social molecular networking (GNPS)

chemicals of emerging concern (CEC)

NORMAN suspect list exchange (NORMAN-SLE)

liquid chromatography hyphenated to mass spectrometry (LC-MS)

gas chromatography hyphenated to mass spectrometry (GC-MS)

ionisation efficiency (IE)

effect-directed analysis (EDA)

Mass Spectrometry Interactive Virtual Environment (MassIVE)

Reanalysis of Data User (ReDU)

Best Practices for Non-Targeted Analysis (BP4NTA)

Study Reporting Tool (SRT)

liquid chromatography hyphenated to high-resolution mass spectrometry (LC-HRMS)

gas chromatography hyphenated to high-resolution mass spectrometry (GC-HRMS)

quality control (QC)

ISO/IEC (International Organization for Standardization/International Electrotechnical Commission)

CITAC (Cooperation on International Traceability in Analytical Chemistry)

quality assurance (QA)

European Environmental Exposure Assessment Research Infrastructure (EIRENE RI)

early warning system (EWS)

International Commission for the Protection of the Rhine (ICPR)

NORMAN early warning system (NormaNEWS)

Prioritisation and Early Warning System (PEWS)

UK Hazardous Substances Advisory Committee (HSAC)

Office of Research and Development (ORD)

United States Environmental Protection Agency (EPA)

EPA Non-Targeted Analysis Collaborative Trial (ENTACT)

Rapid Alert System for Food and Feed (RASFF)

Rapid Exchange of Information System (RAPEX)

European Reference Network for Critical Infrastructure Protection (ERNCIP)

World Health Organization (WHO)

new psychoactive substances (NPS)

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)

European Commission (EC)

European Agency for Safety and Health at Work (EU-OSHA)

Nuclear magnetic resonance (NMR) spectroscopy

Ultraviolet-visible (UV-Vis) spectroscopy

infrared (IR) spectroscopy

European Chemicals Agency (ECHA)

in vitro to in vivo extrapolation (IVIVE)

Horizon 2020 initiative (H2020)

Exposome powered tools for healthy living in urban settings (EXPANSE)

Work Package 4 (WP4)

# 1. Introduction

Characterization of the health risks associated with exposure to hazardous chemicals is paramount for developing preventative measures aimed at reducing risk, alleviating the burden of diseases and improving quality of life. The number of chemicals present throughout food chains, ecosystems and the atmosphere is increasing (Botana, 2016; Chhaya et al., 2022; Noyes et al., 2009; Wang et al., 2020a), raising the chemical burden on the environment and human population (Landrigan et al., 2018; Murray et al., 2020). Systematic characterisation of chemical exposures has been advocated, aiming for a more data-driven approach for prioritization and risk assessment to safeguard humans (Vermeulen et al., 2020) and the environment (Escher et al., 2020; Johnson et al., 2020).

Concerns about the combined effect of exposure to chemicals in mixtures have been widely acknowledged (Drakvik et al., 2020; European Commission, 2012; Kortenkamp and Faust, 2018). Despite these concerns, hazard assessment of individual compounds remains predominant, and a greater examination of chemical mixtures is needed (Bopp et al., 2019; Escher et al., 2022; Heys et al., 2016). Although chemical mixtures have been considered in epidemiological studies for decades, it is rarely with the purpose to inform regulation and risk management (Quiros-alcala and Barr, 2023; Savitz and Hattersley, 2023), and the extent of human exposure to real-life chemical mixtures and associated health risks is mostly unknown (Egeghy et al., 2012; Vandenberg et al., 2023). In addition, risk assessment needs to progress to consider the trajectories of exposure to dynamic chemical mixtures (Pruvost-Couvreur et al., 2020a, 2020b). Finally, the limited number of chemicals widely analysed in the environment means ecological risk assessment is reliant upon chemical emissions modelling (Gustavsson et al., 2023); representing predicted, rather than empirical, environmental concentrations of chemicals.

Delineating the composition of complex chemical mixtures for assessment is highly challenging (More et al., 2019). Notably, industrial property rights can be a hindrance as the identity of manufactured chemicals is often not completely disclosed, and many product mixtures are designated as substances of unknown or variable composition (Lai et al., 2022). Further, many chemicals, natural or manufactured, undergo abiotic and/or biotic transformations during their lifecycle, yet most (bio)transformation products are still to be recognised (Dévier et al., 2011), and the guidance for their inclusion in regulatory risk assessment differs across domains (Escher and Fenner, 2011). Real world chemical exposure burden is likely to be much greater than estimated, and novel methods for the enhanced identification of chemicals of known, emerging or potential future concern and their prioritization for risk assessment are vital.

The European Human Biomonitoring Initiative (HBM4EU, https://www.hbm4eu.eu/) ran from 2017 to 2022 and sought to harmonize European human biomonitoring (HBM) activities and generate evidence to support the EU's environmental and chemical policies with solid scientific data on internal human (aggregated) exposure. The project involved the consolidation of existing human internal exposure data and the implementation of joint studies that aligned ongoing and planned national and regional HBM studies in order to generate comparable data, establish baselines of actual exposure in the European population, and provide evidence to guide regulatory decision-making and policy responses (Ganzleben et al., 2017). Alongside efforts of HBM4EU to harmonise conventional internal exposure characterisation, dedicated activities evaluated the potential to incorporate innovative methodologies into the HBM framework.

HBM4EU's efforts are being continued and extended as part of the European Partnership for the Assessment of Risks from Chemicals (PARC) over the period of 2022 to 2029 (ANSES, 2020; Marx-Stoelting et al., 2023). PARC aims to develop the next generation of chemical risk assessment to protect health and the environment, broadening the scope from HBM4EU to incorporate food and environmental risks (Marx-Stoelting et al., 2023). PARC will use the progress made within the HBM4EU and add new expertise to advance exposure characterisation across the environment-food-human continuum. PARC will apply a combined aggregated exposure pathway (AEP) and adverse outcome pathway (AOP) framework (Price et al., 2020), linking the source, fate, transport and toxicokinetics of chemicals and their potential environmental and human health effects. PARC activities dedicated to innovative methods for enhanced characterisation of exposure to support regulatory chemical risk assessment aim for concerted European advances, but integrating into current environmental and human monitoring frameworks entails new challenges.

We highlight the main barriers that need to be overcome in this process and provide recommendations for building a sustainable European capacity to progress the use of innovative methodologies to improve the characterization of chemical exposure in a regulatory context.

## 2. Barrier 1: Analyte detection & annotation

Traditional chemical exposure characterization is mainly based upon monitoring frameworks established at national and international level. Environmental monitoring and food monitoring are most established, with specified regulatory limits for a wide array of chemicals e.g., polycyclic aromatic hydrocarbons and metals in air e.g., Ambient Air Quality directive (European Commission, 2008a), prioritized substances in water e.g., Water Framework directive (European Commission, 2000), Marine strategy framework directive (European Commission, 2008b), groundwater directive (European Commission, 2006) and the Watch list substances in surface water and groundwater (European Commission, 2018a), and prioritized substances in food for e.g. chemical contaminants (European Commission, 2023a) or residues of pharmacologically active substances (European Commission, 2023b).

While detection of a pollutant in environmental matrices such as air, soil and water indicate a potential exposure agent, proof of exposure occasionally requires detection of the analyte (or a specific biomarker) in biospecimen (internal exposure). On the other hand, detection of internalized exposure agents in humans and biota is rarely indicative of the exposure source and therefore of limited value for the development of exposure reduction and mitigation strategies. Human and environmental (bio)monitoring is still in need of significant improvements (Zare Jeddi et al., 2022) to further our understanding of the source-internal exposure continuum. Few chemicals have HBM-based guidance values (HBM-GVs) for the general population (e.g., blood lead concentrations) and regulations containing maximum internal human exposure levels are restricted to the occupational domain. Yet, even in occupational settings HBM is underutilised and ambient air monitoring and maximum ambient air concentrations prevail over biomonitoring (Viegas et al., 2020).

Traditional monitoring frameworks involve the quantitative measurement of a limited number of prioritised substances. These frameworks are not designed for comprehensive determination of other chemicals of potential concern. Complementary, exploratory methods to screen a broader chemical space and enable the rapid identification and prioritization of potentially harmful chemicals associated with exposure are required.

Mass spectrometry (MS) is a platform of choice for chemical analysis due to high sensitivity and possibility for qualitative (i.e., detection of known compounds and structure elucidation of unknown compounds) and/or quantitative analysis. When analysing complex chemical mixtures, MS is typically coupled to gas or liquid chromatography (GC or LC) for preceding separation of analytes. Commonly, approaches for chemical measurement are divided into targeted, suspect and non-targeted, typically related to the status of analyte identification and coupling quantification (Krauss et al., 2010; Pourchet et al., 2020). Since feature identification and quantification are fundamentally independent, we favour division based upon the mode of MS data acquisition:

Targeted data acquisition operates a user-defined selective scanning in the first domain (MS1). Selected target ions are predefined and can be specific for known chemicals or diagnostic of substructures of interest. Non-targeted acquisition operate a non-selective ion acquisition in MS1 (Table 1). Both approaches can provide qualitative i.e., presence of analyte; and/or quantitative information.

By design, selective ion acquisition approaches, such as selected ion monitoring (SIM) and multiple and parallel reaction monitoring (MRM and PRM, respectively), typically achieve higher specificity, selectivity and sensitivity than non-selective procedures, to provide lower limits of detection and quantification (LODs & LOQs). Following user input of precursor ions (as per SIM), MRM method development can be computationally expedited, with the automated selection of e.g., transition ions, collision energies, dwell and cycle times following the analysis of authentic standards. For example, quantification via stable isotopic dilution approach for >1000 chemicals contaminants in feed via LC-MS/MS (MRM acquisition) has been demonstrated (Steiner et al., 2020). Alternatively, MRM transitions can be selected from detected features observed from full-scan analysis or even predicted, exampled for both LC (García-Reyes et al., 2007; Sawada et al., 2009) and GC (Li et al., 2012; Yuan et al., 2022) coupled separations. Similarly, PRM methods that acquire MS2 spectra can be established quickly due to no need to optimise transitions, with greater selectivity favouring a reduction in reliance on chromatographic separation. However, the slower scan speed limits throughput, especially hindering the measurement of analytes in opposing polarity ion modes in a single assay (Zhou and Yin, 2016). These approaches showcase that the routine, reliable detection of hundreds of analytes, including those tentatively identified, is feasible.

By comparison, data generated by non-targeted methods are not limited to user-defined precursor ions. As such, non-targeted approaches enable the detection of analytes and/or substructures not fully defined *a priori*. The most commonly applied acquisition approaches for non-targeted methods are full-scan collecting MS1 data only, or additionally collecting product ion spectra in higher domains with or without precursor ion selection (data-dependent and data-independent, respectively). For example, precursor ion scans operate whereby MS1 is scanned and MS2 ions user-prescribed and all detected features will share a common defined substructure. Precursor-ion scanning methods have been widely applied e.g., to detect unknown anabolic steroids during antidoping testing (Pozo et al., 2008) and the number of analytes detected will be unknown and not fully dependent on user input.

Lastly, we deem suspect screening approaches to be a subset of non-targeted procedures, whereby the resultant data is then selectively analysed using user-defined ions that are specific for known chemicals (Krauss et al., 2010), or diagnostic of substructures of interest i.e., predetermined, rather than de novo, spectral interpretation.

Non-targeted acquisition approaches enable combined detection of a priori known analytes with detection of analytes not pre-defined and simultaneous quantification and discovery. For example, the combined absolute quantification of 80 organic contaminants, suspect detection of 69 analytes and structural elucidation with subsequent confirmation of 1,3-benzothiazole-2-sulfonate was demonstrated by non-targeted acquisition of wastewater samples (Schymanski et al., 2014b). Moreover, the quantification and suspect detection of a wide range of pesticides in a variety of food samples using a single measurement has been validated (Zomer and Mol, 2015).

Table 1. Overview of MS scan types for targeted and non-targeted acquisition.

| | **MS1 scan type** | **MS1 generic approach name** | **MS2 scan type** | **MS2 generic approach name** |
|---|---|---|---|---|
| **Targeted** | Selective | Selected ion monitoring (SIM) | SIM | Selected reaction monitoring (SRM) |
| | | | MS filters[a] | target-directed data dependent acquisition (tDDA) |
| | | | Fs | product ion scanning / parallel reaction monitoring (PRM) |
| **Non-targeted** | Scan | Full MS / Full scan (Fs) | SIM / neutral loss (NL)[b] | precursor ion scanning / neutral loss monitoring (NLM) |
| | | | MS filters | Fs data dependent acquisition (DDA) |
| | | | Fs | Fs data independent acquisition (DIA) |

[a] Ions are selected by MS

[b] Though operating a scan, offset is user-selected

A key benefit of non-targeted acquisition is that the generated data can be archived and re-analysed as more information and resources become available in future. Within the HBM4EU Survey on Pesticide Mixtures in Europe (SPECIMEn) study, a suspect screening focussed upon matching isotopic pattern recognition-based formulas generated per feature to a curated database of >4600 pesticides and their potential metabolites in urine, resulting in confirmation of 14 parent pesticides (Huber et al., 2022). Within PARC, the SPECIMEn full-scan dataset will be further mined to detect and identify other chemicals of concern. Further still, members of the Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances (NORMAN) conducted a suspect screen of >2000 emerging contaminants in wastewater with retrospective absolute quantification of 395 detected analytes via standard addition calibration (Gago-Ferrero et al., 2020), whilst the retrospective quantification of 20 parent azole antifungals and three (bio)transformation products in various matrices of aquatic ecosystem (e.g., surface water, soil, gammarids, fish) to calculate partitioning and bioaccumulation factors (Creusot et al., 2020).

Non-targeted acquisition methods can provide information for a far broader array of chemicals than targeted measurements. Most non-targeted approaches are based on high-resolution mass spectrometry (HRMS), which aids the identification and structural elucidation of unknowns (Hollender et al., 2017; Sandra et al., 2016). Therefore, non-targeted approaches enable exploratory insight into the chemical composition of samples, which is paramount to detect contaminants of emerging

concerns and identify new chemical risks. However, the ambiguity of identification presents new challenges for risk mitigation when informing the risk management context and decision makers.

*Coverage*

The vastness of potential chemical exposure agents means that defining the compositional space of hazardous chemicals is imperative to focus detection and identification efforts. While boundaries for chemical space of halogenated organic compounds with high bioaccumulation potential or persistence have been defined, the bounds for non-halogenated organics are challenging to delineate (Zhang et al., 2019). Compared to human metabolites, greater proportions of parent xenobiotics are cyclic, achiral, contain halogen and/or sulphur atoms and/or have secondary and/or tertiary amine functional groups. Moreover, ~85% have a log P between −5 to +5, whereas > 55% of metabolites are above this range (Khanna and Ranganathan, 2009). However, xenobiotic datasets are often skewed towards legacy chemicals and the breadth of chemical space for environmental contaminants, including chemicals of emerging concern, is underrepresented (Grulke et al., 2019). Safe-by-design principles (European Commission, 2022) will likely lead to greater convergence of properties in future and though safer, substances newly introduced in commerce more challenging to distinguish and thus elucidating mechanisms underlying adverse effects may become harder.

No single analytical approach or platform can measure the complete chemical composition of a sample and it is imperative that the development of new methodologies (from sample preparation to annotation) is stimulated. Notably, implemented HRMS based applications to screen for xenobiotics were reviewed to be limited and recommendations to enhance detection coverage prioritised (Vitale et al., 2021). Furthermore, a framework to map the coverage of chemical space that any given untargeted analytical methodology can detect has been proposed (Black et al., 2022), with hopes of informing method development performance evaluation and to increase confidence in communication of results yet moving from framework to tangible implementation is challenging.

*Annotation*

Annotation is the process of linking a detected mass spectrometric feature with a chemical identity, considering the detected chromatographic and spectrometric characteristics. The reference data used for suspect annotation can be based on empirical or predicted data, confining, or expanding the chemical space depending on application requirements.

Annotation may lead to identification if sufficient analytical evidence can be provided that the compound is indeed the proposed chemical. Identification is generally accomplished by comparing measured data sets, where one set of features is obtained from the analysis of an unknown compound, the other from a reference standard of known identity. Reference data might be retrieved from curated databases.

The number of openly available reference spectra of xenobiotics and their derivatives remains limited. For example, EU MassBank, an open-access spectral database focused upon pollutants (Schulze et al., 2012), contains spectra from > 16,000 chemicals, a limited fraction of the number of chemicals registered for commerce (Wang et al., 2020b). This has been recognized and lists of marketed chemicals with tonnage data as well as mass spectral data and reference standards have been requested from industry to facilitate the annotation of industrial chemicals (Hollender et al., 2019).

Notably, the Global Natural Products Social molecular networking (GNPS) platform has rapidly advanced natural product dereplication efforts (Wang et al., 2016) through community sharing of acquired MS datasets. GNPS molecular networking is increasingly being applied to investigate xenobiotics e.g., in air (Papazian et al., 2022) and water (Oberleitner et al., 2021; Petras et al., 2021) samples, and increased public deposition of datasets derived from studies aiming to assess chemical exposure could similarly increase annotation rates.

In addition to empirical records, MS ready related information databases and predicted records are widely used. The prediction of (bio-)transformation/reaction products of known chemicals is commonly done to expand suspect chemical space and recently a database integrating > 2 million natural and synthetic chemicals into a global network recently demonstrated (MohammadiPeyhani et al., 2022). Within the HBM4EU project, the CECscreen database was developed via prediction of phase I biotransformation products of chemicals of emerging concern (CEC) (Meijer et al., 2021). CECscreen was incorporated into the NORMAN suspect list exchange (NORMAN-SLE), a compendium of similar records that already facilitates suspect screening of chemicals amenable to LC-MS and GC-MS (Mohammed Taha et al., 2022). For the environment, microbial biotransformation products have been included from registration dossiers or prediction systems like enviPath (Wicker et al., 2016).

Searching for a small number of suspects can result in low coverage of the chemical space, however large numbers can lead to redundant matching and high numbers of candidates per feature. Therefore, structural candidates require filtering via incorporation of additional information, such as retention times/indices and other physicochemical properties e.g., collision cross section values from ion mobility. Context-specific libraries and databases have been shown to enhance annotation rates (Gauglitz et al., 2022) and greater enrichment of metadata in spectral libraries advocated to improve identification.

## 3. Barrier 2: Analyte quantification

Although the main bottleneck of exploratory analyses is the progression from preliminary annotations to confirmatory identification and structural elucidation of observed features (da Silva et al., 2015), the quantification of detected substances is also required to assess the extent of exposure. When a reference standard is available, both confirmatory identification and the estimation of concentration in samples is relatively straightforward, i.e., direct comparison of properties and analytical response of the observed features and the authentic chemical standard. However, for many chemical agents and their derivatives, reference standards are not yet available and thus the identification and quantification of an analyte from exploratory screening methods is complex.

Whereas concentration estimates of identified analytes with a defined measurement uncertainty, can be directly interpreted with respect to numerically definable safety thresholds, the output of exploratory approaches is typically qualitative or relative quantification. While this permits inter-sample comparisons of exposure patterns, the interpretation and communication of such data are challenging. Consequently, absolute quantitative analyses remain required for exposure risk assessment and to support risk management decision making (Mccord et al., 2022a).

Nonetheless, procedures to estimate concentrations from non-targeted acquisition methods have been developed and some can be extended to all detected features e.g., bounded response-factor method or annotated features, e.g., compound ionization response modelling (Mccord et al., 2022b). Typically, the concentrations of 90% of detected features can be predicted within two orders of

magnitude, yet the extrapolation of estimated concentrations observed to true environmental or biological concentration depends on the sample preparation procedure used for the matrix (Liigand et al., 2018). Notably, ionisation efficiency (IE)-based procedures for electrospray ionisation were shown to be more accurate than approaches based on comparison to analytes with similar retention time or structure (Kruve et al., 2021). Further still, comparable accuracy has been evidenced for (IE)-based prediction of concentrations using structural information and when relying on data-derived descriptors without structural assignments, extending the approach to quantify unidentified features (Palm and Kruve, 2022). That said, IE prediction models require training on large collections of empirical ionisation response data for analytes representative of intended chemical space and limited data is currently available, hindering accuracy and applicability.

Although the concentration of unknown features is disconnected from hazard risk, it can support feature prioritisation for subsequent identification. Furthermore, estimated concentrations can be informative when unknown features are assigned to the same class as known and/or regulated hazardous chemicals e.g., phthalates in food packaging (Pieke et al., 2017). At this stage, estimating feature concentrations in non-targeted analysis is complex and associated to significant uncertainty and not widely implemented but shows promise to bridge the gap between conventional monitoring and exploratory approaches for chemical exposure characterisation.

## 4. Barrier 3: Analyte prioritization

The tentative annotation of hundreds to thousands of features means prioritisation is required to guide follow-up investigation, with aims to focus on features of greatest risk to health and environment.

It has been argued that high persistence in the environment and the potential for bioaccumulation give rise to specific concerns, especially as their long-term effects are difficult, if not impossible, to predict due to temporally and spatially de-coupling of emissions and impact. Once they have entered the environment, the presence of these chemicals is difficult to reduce. Frequent detection of a persistent chemical in multiple environmental matrices hence raises concern, irrespective of observed detrimental impacts (Cousins et al., 2019). Following on from this approach, high bioaccumulation should similarly raise concern, and frequent detection of a chemical in multiple biotas including relevant sentinel environmental media and animal species should be flagged, independently of observed adverse outcomes. This approach is pertinent for chemicals of emerging concern that typically lack toxicity data.

Features are often assigned with multiple possible structures, candidates for confirmatory analysis could be based upon the prediction estimates of these two properties for all possible entities, with a precautionary approach assuming the greatest persistence and bioaccumulation. Furthermore, these characteristics can be used for the selection of unidentified features to be prioritized for structural elucidation. For example, based on increasing temporal trends in time-series samples of abiotic and biotic matrices (Plassmann et al., 2018), indicating high persistence and high bioaccumulation, respectively. The analysis of multiple trophic levels of a food web extends the approach to biomagnification (Fu et al., 2022) and similarly, sampling at multiple sites to identify spatial trends, e.g., in aqueous media to extend for chemical mobility.

Alternatively, probabilistic hazard quotients, based on predicted human exposure and estimated toxicity, can prioritize exposure agents with higher probabilistic health risk estimates (Zhao et al.,

2021), or potential toxicity could be directly implied from predicted MS2 spectral substructure assignments, without exact structural elucidation (Arturi and Hollender, 2023; Peets et al., 2022). However, these models rely on training upon large collections of previous toxicological data, ideally for related analytes, which are often deficient and so limits accuracy and transferability.

*Bioactivity-based prioritisation*

Instead of a reliance on predicted biological and toxicity properties, effect-based methods use *in vivo* and/or *in vitro* tests (Brack et al., 2019) to measure the biological activity of samples. The coupling of effect-based methods with chemical analysis enables the prioritisation of features based on their bioactivity using effect-directed analysis (EDA) (Simon et al., 2015) and is widely applied to complex environmental mixtures, food, and biota samples to assess unknown effects and identify risk drivers.

Feature prioritisation in EDA is based on the premise that only a fraction of the components of complex mixtures significantly contributes to an observed adverse effect (Brack et al., 2016). To this end, samples are separated into fractions, often using preparative LC, and tested for bioactivity using a combination of assays measuring toxicological endpoints. Feature identification via non-targeted analysis is then focused on fractions that show greatest activity, reducing sample complexity to expedite identification of hazardous chemicals.

Several so far unknown drivers of toxicity and risk have been identified in surface waters using EDA including, for example, the potent antiandrogenic fluorescent dye 4-methyl-7-diethylaminocoumarin (Muschket et al., 2018), several antidepressants and anthelmintic agents impacting on sea urchins in the coastal zone (Mijangos et al., 2020) and 2-anilino-5-(4-methylpentan-2-ylamino)cyclohexa-2,5-diene-1,4-dione (6PPD-quinone) as the toxicant primarily responsible for urban runoff mortality syndrome affecting coho salmon in the US (Tian et al., 2021).

The correlated occurrence of analytes observed between biotic and abiotic matrices, especially across ecological networks and consumer-resource systems can support the identification of analyte-analyte relationships, aiding structural elucidation and to understand chemical fate and transport, paramount for source identification. In the case of 6PPD-quinone, the unidentified feature was prioritised based on detection in roadway runoff water and tire tread wear particle leachates alongside greater concentrations measured in runoff waters occurring during storms with documented salmon mortality. Knowledge of the precursor, 4-*N*-(4-methylpentan-2-yl)-1-*N*-phenylbenzene-1,4-diamine (6PPD) being used as a tire rubber additive (Engels et al., 2000) supported causal source identification, leading to calls for increased regulation of tire wear particles (Trudsø et al., 2022).

A key benefit of EDA is that it can focus investigations on unidentified features that account for the gaps in expected and observed biological effects of chemical mixtures (Altenburger et al., 2019) through (i) bioactivity comparisons between reconstituted mixtures composed of chemicals identified in each sample or fraction with total fraction/sample (ii) mass-balance calculation consisting in the comparison of the actual biological activity to the predicted one based on the chemical quantification of known active chemicals with known potency (Brack et al., 2016; Neale et al., 2015). However, the major drawback of EDA is the intensive and time-consuming nature of assaying multiple fractions, which hinders application for large scale studies. Downscaling of assays and high-throughput fractionation (Zwart et al., 2018), or parallel fractionation and non-targeted MS acquisition (Jonkers et al., 2022) may help to at least partly mitigate these restrictions. Furthermore, when assaying non-

specific toxic endpoints, the prerequisite that effects are based on only a fraction of chemicals is often not achieved and although using assays for specific endpoints alleviates this, it then restricts prioritisation to selected endpoints. Finally, EDA is typically conducted on sample extract with *in vitro* tests and so the bioassay results often do not reflect toxicant bioavailability (Burgess et al., 2013). Nevertheless, the development of miniaturized *in vivo* effect-based methods and/or associated calculation of effect-based trigger values through comparison with *in vitro* is promising (Brion et al., 2019).

## 5. Barrier 4. Reporting

The diversity of approaches applied for characterisation of chemical exposure means that frameworks for unified reporting are vital for communication to stakeholders.

Building upon initial minimal reporting standards (Sumner et al., 2007), updated recommendations and a reporting checklist for mass-spectrometry based small molecule analysis have been developed (Alseekh et al., 2021; Kirwan et al., 2022). These guidelines are intended to complement and extend requirements implemented by data repositories like MetaboLights (Haug et al., 2020) (https://www.ebi.ac.uk/metabolights/), Mass Spectrometry Interactive Virtual Environment (MassIVE) (https://massive.ucsd.edu/) and Metabolomics Workbench (Sud et al., 2016) (www.metabolomicsworkbench.com), and meta-data analysis systems, such as the Reanalysis of Data User (ReDU) framework of GNPS (Jarmusch et al., 2020). Furthermore, the US-based working group Best Practices for Non-Targeted Analysis (BP4NTA), established a Study Reporting Tool (SRT) for evaluation of the quality and completeness of non-targeted data reporting (Peter et al., 2021). Unlike previous reporting checklists, the BP4NTA SRT was designed with reviewers and editors in mind, to aid them in assessing the comprehensiveness, reproducibility, and transparency of reporting, showing greater potential for enforcement via journals and could also aid in strengthening communications in the context of regulatory chemical risk assessment.

In particular, the provision of reliable information on confidence in annotations and quantification of analytes is essential as regulatory measures to reduce the risks associated with exposure to chemicals, such as reduction or restriction of production, can result in important economic and societal consequences (Rivier, 2003).

*Identification confidence*

Various criteria are available for monitoring of *a priori* known substances to ensure accurate reporting, via confirming observed physicochemical properties of measured analytes with those of authentic reference standard. However, in exploratory analysis the detected features are annotated post data acquisition. Structural elucidation is a stepwise process reliant on multiple lines of evidence and as such, detected features are annotated to differing degrees.

Identification scales for non-targeted approaches are often based upon the original minimum reporting standards proposed via the Metabolomics Standards Initiative (Sumner et al., 2007). Subsequently, Schymanski et al. updated the scale to cover the increased possibilities of HRMS (Schymanski et al., 2014a) and it is now the most widely adopted. Numerous other periodic updates have been proposed for the greater inclusion of technological advancements e.g., LC-HRMS (Jeon et al., 2013), GC-HRMS (Koelmel et al., 2022), ion mobility spectrometry (Celma et al., 2020; Schrimpe-Rutledge et al., 2016), or specific chemical classes e.g., PFAS (Charbonnet et al., 2022). Additionally, an

extension to incorporate lipid notation and more detailed reporting of isomers was proposed (Rampler et al., 2021).

In addition, more extensive systems to assign points for annotation of analytes have been proposed, predominantly based upon prior versions of EC criteria designed for the detection of a priori identified veterinary drugs in food and feed (European Commission, 2021). For instance, a quantitative scoring framework incorporating multiple analytical platforms including HRMS was proposed in 2014, but the complexity noted as a drawback and limit to uptake at the time (Sumner et al., 2014). That said, the quantitative metrics were used as the basis of a quality control (QC) system to integrate various datasets for meta-analysis, evidencing feasibility (Buendia et al., 2019). Later, extended points scales to communicate identification specific for LC-HRMS were proposed, such as the Rochat scale that includes extensive additional information like occurrence probability (Rochat, 2017), or a recent scale that only used objective criteria to enable automation and was mapped to the Schymanski scale for ease of uptake (Alygizakis et al., 2023).

Even though a plethora of scales are available, their use is not consistently reported (Salek et al., 2013), and even for the relatively simple minimum reporting standards originally proposed by the MSI, they are not correctly adhered to (Kodra et al., 2022; Theodoridis et al., 2023). More complex and information rich scales are needed in a regulatory context and so the focus should be on enhancing the adoption and adherence to new propositions.

*Non-targeted acquisition method performance assessment and quality assurance / quality control*

In addition to analyte identification, the availability of reference chemical standards enables the measurement of key analytical parameters, such as trueness and precision, selectivity, limits of detection and quantification and uncertainty. As such, the overall performance of targeted assays can generally be reported with respect to all measured analytes. However, evaluating the performance of non-targeted approaches is more complex.

While ISO/IEC (International Organization for Standardization/International Electrotechnical Commission) 17025 and ISO 15189 accredited laboratories are required to be able to report valid qualitative results, there is no formal need to report the uncertainties associated with qualitative analysis. Despite recognition that formal reporting standards often do not exist, Eurachem/CITAC (Cooperation on International Traceability in Analytical Chemistry) guidelines state that it is realistic to expect most laboratories to adequately control the relevant parameters of qualitative analysis procedures and provide only a minimal recommendation ‘to check at the least the most critical false positive rate’ (Bettencourt da Silva and Ellison, 2021).

It was discussed that nothing in the ISO/IEC 17025 framework is incompatible for non-targeted approaches and that adaptations could be made for implementation as formalised quality assurance (QA)/QC (Monteiro et al., 2021). The emphasis was on harmonization of QA procedures and the authors noted that adaptations for adoption would require wider consultation.

To date, evaluations of non-targeted approaches are not unified, and a variety of approaches undertaken (Bastian et al., 2020). QC samples spiked with known concentration of reference standards covering a specific polarity and mass range are often used. In many cases, the reference standards are stable isotopically labelled and furthermore added to all samples to correct for e.g., matrix effects and

retention time drifts (Caballero-Casero et al., 2021). Such an approach was exampled within the HBM4EU SPECIMEn study, whereby monitoring of detection of labelled markers in QC materials was implemented prior to sample analysis to ensure data acquisition was above a threshold quality (Vitale et al., 2022). Alternatively, well-characterised reference matrix materials, such as Certified reference materials (CRMs) can be used where available, as evidenced for e.g., dust (Newton et al., 2020). However, because there is no ground truth for analytes present in a sample, these procedures focus more on assuring detection of known chemicals and do not assess the overall performance of feature detection and/or analyte identification.

In this regard, use of a confusion matrix to calculate true positive rates, false negative rates, precision and false discovery rates for assessing identification has been proposed (Fisher et al., 2022). However, the metrics require analytes to be annotated with a single structure and so are only applicable to features defined at high confidence, being dependent on a high degree of initial sample characterisation and/or number of spiked analytes. Notably, a defined mixture of 89 electrospray ionization amenable standards with diverse physicochemical properties was developed to provide a QC control mixture to evaluate method adequacy and can be incorporated into workflows to enable standardised comparative quality control assessment (Knolhoff et al., 2021). Yet, since most analytes are native (in part due to cost viability and limited availability of labelled standards), applying the mixture to evaluate quantitative performance is complex. Moreover, the mixture composition was designed to encompass a broad chemical space and may be of limited use for assessing more focused methodologies, e.g., suspect screening of specific chemical classes.

Moreover, no requirements are imposed on computational methodologies and applications for data analysis. A lack of quality standards for software hinders reproducibility, even in the minority of cases that versions and parameters used are reported (Considine et al., 2018). A shift towards platforms designed for reproducible analysis, e.g., via software containers (Perez-Riverol and Moreno, 2020) and workflow engines, such as Galaxy (Afgan et al., 2022) has been advocated (Chang et al., 2020). Notably, Galaxy has been elected the environment of choice for hosting HRMS processing pipelines within the European Environmental Exposure Assessment Research Infrastructure (EIRENE RI; https://www.eirene-ri.eu/), favoured to leverage, extend and cooperate with an established developer and user community for mass spectrometry-based exposomics and metabolomics data processing.

Establishing and adhering to harmonised QA/QC criteria, metrics and frameworks for performance evaluation and transparent reporting are essential to ensure comparability, reproducibility, and validation of results. It has been noted that even for publicly available datasets there is low compliance with recommended reporting guidelines (Spicer et al., 2017). Critically, developed guidelines must be enforceable and interpretable by decision-makers.

## 6. Barrier 5: Using European monitoring to support an Early Warning System

The identification and prioritisation of potentially hazardous chemicals are critical components of EWS to mitigate exposure risks. Yet, there is limited guidance for the deployment of exploratory approaches within EWS to detect and evaluate risks of exposure to hazardous chemicals for the general population. One example of early warning tools are non-target screening approaches. The application of non-targeted approaches is better established in the fields of environment and food, although inclusion in EWS is currently limited to the provision of information on the presence of chemicals. Concerted

efforts will be required to align the implementation of innovative methodologies for exposure characterization at EU-wide scale, and especially extension to human monitoring.

*Landscape of innovative methodologies in national EWS*

When conducting non-targeted acquisition, reporting of analytes with confirmed identification and absolute quantification can follow guidelines for targeted assays. Beyond this though, few countries have incorporated exploratory analysis into regulatory frameworks for human, food and/or environmental monitoring for chemical risk assessment.

Most notably, an EWS incorporating non-targeted analysis has been implemented along the Rhine River for ~10 years (Hollender et al., 2017; Ruff et al., 2015). Building upon this, in 2021 the International Commission for the Protection of the Rhine (ICPR) commenced a project to extend this approach to an additional three environmental authorities in Germany and one in the Netherlands. The envisioned approach is similar to that demonstrated via the NORMAN EWS (NormaNEWS), whereby features detected in one laboratory are fed into the retrospective suspect analysis of data generated at other laboratories (Alygizakis et al., 2018). Consequently, occurrence rate information can be extended to a far larger body of data in a rapid manner. Furthermore, the incorporation of non-targeted analysis and effect-based methods into World Health Organisation (WHO) Water Safety Plans (World Health Organization, 2022a) has been recommended (World Health Organization, 2022b).

The Environmental Agency in the UK has recently developed a Prioritisation and Early Warning System (PEWS) for England. The system is intended for identification of chemicals of concern in the environment to establish a base for risk assessment and ensure appropriate regulatory focus. Currently, PEWS is based upon readily available data, yet there are future plans to include NTA to provide presence/absence information (Sims, 2022), with methods currently under development. The UK Hazardous Substances Advisory Committee (HSAC) strongly supported the PEWS initiative and explicitly recommended knowledge exchange, data sharing and cooperation with PARC (Hazardous Substances Advisory Committee, 2021).

Similarly, the Office of Research and Development (ORD) of the United States Environmental Protection Agency (EPA) have outlined a structure for the incorporation of non-targeted analysis to support identification and risk-based chemical prioritisation that bridges high-throughput exposure and bioactivity evaluation (Sobus et al., 2018). Non-targeted acquisitions are used to support screening activities whilst targeted, quantitative data are used for risk-based decision making. Following this integrated framework, the EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) was launched to stimulate the advance of non-targeted approaches for characterising xenobiotics in environmental and biological matrices. ENTACT is operating a defined ring-trial to enable comprehensive performance evaluations alongside enhancing the resources available through provision of defined chemical mixtures, suspect lists, and reference mass spectra to the community (Ulrich et al., 2019). Outputs of ENTACT are expected to guide future non-targeted acquisition method development strategies and inform on gaps for applications in decision making by gauging the current validity and reliability of data reported. Notably, the ORD advocated for standardized approaches for generation, reporting and use of non-targeted data (Sobus et al., 2018).

*European capacity*

In addition to the national frameworks, an EWS to control food safety, a Rapid Alert System for Food and Feed (RASFF) has been set-up to facilitate rapid reaction by food safety authorities to public health risks arising from the food chain and to ensure the exchange of information between member states. It is legislatively based on the General Food Law (European Commission, 2002). Furthermore, the Rapid Exchange of Information System (RAPEX) is an EWS for rapid alerting of unsafe consumer products and consumer protection but does not include food and pharmaceuticals (European Commission, 2018b). Both systems work to ensure the early identification of chemical hazards in food and non-food products.

The European Reference Network for Critical Infrastructure Protection (ERNCIP) Water Security Plan (Ribas Batlle et al., 2022) includes non-targeted screening approaches within an EWS to assess toxicity and chemical contaminants detection of breaches to water quality.

Regarding human biomonitoring, an EWS for new psychoactive substances (NPS) has been operated by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) since 1997, established on legislative framework for risk assessment of NPS (EMCDDA, 2019). A network of national EWS regularly reports newly identified psychoactive substances to the EMCDDA, which collects and collates the data to assess potential risks for health or society at the EU-level. Based on the initial report, the European Commission (EC) can request a comprehensive risk assessment to be undertaken for specific NPS and prepare potential control actions to be implemented at national and EU levels. While qualitative screening of NPS via non-targeted acquisition is already established in national forensic laboratories, the procedures and approaches vary between each laboratory and the population exposure risk remains challenging to estimate (Shafi et al., 2020). Furthermore, like pharmaceuticals, NPS are actively taken by a subset of the population, easing detection and identification efforts compared to the passive exposure of the general population to a broad range of chemicals (David et al., 2021).

While 14 EU countries have EWS in place for the monitoring of occupational chemical risk, these rely on the reporting of health events (case reporting and related occupational risks) and do not include prospective monitoring of potentially hazardous chemicals in workers (Palmen, 2016). The lack of adequate exposure assessment was highlighted as one of the obstacles to the implementation of occupational alert and sentinel approaches, although recognized the importance especially in terms of identifying potential new or emerging work-related diseases (European Agency for Safety and Health at Work (EU-OSHA), 2017). No EU-wide alert and sentinel surveillance system is on the political agenda but could contribute to evidence-based prevention and policy, in line with a vision Zero approach to work-related deaths and diseases.

Establishing an EWS for population risk to chemical exposure lags further behind. In part, this is due to limited capabilities for large-scale measurements. For example, despite establishing a network of 75 qualified laboratories, 30% of the >40,000 targeted, quantitative measurements of select priority substances within the HBM4EU project were handled in just three laboratories (The Trace Analytical Laboratory, RECETOX, Masaryk University, Czechia; Department of Growth and Reproduction, Rigshospitalet, Denmark & the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Ruhr-Universität Bochum, Germany) (European Human Biomonitoring Initiative, 2022). Moreover, for some substances the implementation of a decentralised network of

laboratories hindered data comparability and aggregation, particularly when laboratories reported considerably different detection frequencies (Vorkamp et al., 2023). The challenges of decentralised measurements will become even more pronounced for non-targeted approaches because without defined limits of detection, even occurrence rates of features will be more challenging to compare. Furthermore, despite potential for harmonised, multi-site suspect screening procedures being evidenced, e.g., SPECIMEn (Ottenbros et al., 2023), the complexity of associated data processing remains a bottleneck.

## 7. Scientific recommendations to advance use of innovative methodologies for regulatory risk assessment

Overcoming Barrier 1: Analyte detection & annotation

The development and use of complementary, orthogonal methodologies are required to enhance the coverage of chemicals detected. More diverse sample preparations and chromatographic separations should be applied, leveraging novel sorbents and column phase chemistries for enrichment/depletion, purification and chromatographic deconvolution. Greater consideration should be paid to the trade-off between the generic sample preparation and masking of lower abundant analytes. Additionally, technological improvements, such as increasing the intra-scan dynamic ranges of HRMS platforms and microfluidics interfacing are vital to improve sensitivity. Furthermore, multiple ionization modes should be employed when using MS detection and the integration with other measures of physicochemical properties (e.g., NMR, UV-Vis or IR spectral data, ion and electrophoretic mobility etc.) promoted.

As evidenced within the natural product and metabolomics communities, greater sharing of MS data and reference spectra enhances annotation capabilities through leveraging community-wide knowledge and development. Major efforts have been made on the progress of suspect list databases, however, the deposition of raw data and spectra from studies focused on the analysis of environmental chemicals is lacking. In addition to motivating researchers to deposit their data, regulatory bodies, such as European Chemicals Agency (ECHA) should incentivize the deposition of reference mass spectra and related chemical properties data at the point of registration by producers.

Overcoming Barrier 2: Analyte Quantification

The accuracy and uncertainty of the estimation of absolute concentrations from non-targeted approaches requires further development, evaluation and feasibility testing, particularly with respect to reporting concentrations for tentatively identified or unidentified features (Table 2), and potential implications for data misuse / miscommunication. A repository for community provision of ionisation efficiency data has been set up and collection of information about ionisation efficiency should be implemented into reference mass spectral databases (Liigand et al., 2021).

Table 2. Summary of approaches for estimation of absolute concentration for non-targeted acquisition MS analysis of chemicals.

| Identification status | Approach | Relative quantitative uncertainty | Feasibility |
|---|---|---|---|
| Structure assigned | Reference standard | Low | Low |
| | Structure-based IE[a] prediction | Medium | High |
| | Surrogate standard | Medium | Low |
| Unidentified feature | Non-structural descriptor-based IE predictions | Medium | High |
| | Method response bounding | High | Medium |

[a] Ionisation efficiency

Overcoming Barrier 3: Analyte prioritization

A framework for feature prioritization strategies specific for various contexts should be developed. For example, a tiered prioritization based on i) estimated persistence and bioaccumulation and ii) probabilistic hazard quotients and reported occurrence information could be developed for identified features.

Multi-matrix and spatiotemporal studies, particularly across different domains of the environment-food-human continuum, should be increasingly implemented to enable prioritisation of unconfirmed or unidentified features via e.g., time-trends, spatial trends and matrix co-occurrence and co-correlation patterns. Notably, greater integration of internal and external chemical measures will benefit source identification.

The throughput of effect-based methods should be increased and more (eco)toxicity endpoints incorporated into EDA procedures, especially through the parallelisation of fractionation and miniaturisation of *in vitro* and *in vivo* assays. Also, since most effect-based methods are human based, more environmental and ecologically relevant assays should be developed (e.g., fish nuclear receptors, microbial-community based assays). The incorporation of assays accounting for bioavailability, as well the as the implementation of *in vitro* to *in vivo* extrapolation (IVIVE) approach will be needed to increase the relevance of feature prioritisation based on toxicity. Finally, because EDA is time consuming, a two-step strategy consisting of in (i) mass balance calculation, allowing to unravel if know chemical explained the detected activity, and then (ii) fractionation seems relevant.

Overcoming Barrier 4: Reporting

A uniform identification scale should be used to report the confidence and reliability associated with reported chemical identities. The scale needs to be sufficiently detailed, objective, and applicable for automation. Aids for interpretation of the scale and communication should be provided for stakeholders and decision makers.

Harmonised data processing and annotation workflows should be adopted to support comparability of generated results. This is especially vital for multi-centre and multi-platform studies. Additionally, techniques to integrate and fuse sparse, discordant datasets of MS measures of chemicals via analytical methods with different chemical space coverage need development and models to link data from different sample sources across the environment-food-human continuum required.

A harmonized method performance assessment reporting tool should be adopted, with the enforcement of minimum reporting requirements including method adequacy, quantification estimation uncertainty, coverage and detection ranges. Adhering to these reporting requirements promotes transparency, reproducibility, and comparability of results across different studies and laboratories.

Overcoming Barrier 5: Scaling to a European Early Warning System

Non-targeted analysis and EDA are important early warning tools within an EWS. A minimum identification level for reporting detected substances to an Early Warning System (EWS) or regulatory entity should be established to ensure that only confirmed, or sufficiently identified substances, are escalated.

The feasibility to integrate non-targeted analysis and EDA into EWS frameworks requires evaluation with respect to the effectiveness to enhance capabilities for early detection and warning for chemicals of presently emerging and potential future concern.

A network of harmonised qualified monitoring laboratories should be implemented to provide capacity for large-scale monitoring. Select analytical workflows should be standardised as much as possible to enable routine generation of comparable datasets, as per traditional monitoring. Such a network can build upon the HBM laboratory network developed during the HBM4EU and also the NORMAN network. Furthermore, building on cooperations within the Horizon 2020 initiative (H2020) project Exposome powered tools for healthy living in urban settings (EXPANSE), the EIRENE RI has commenced a pilot EU-US harmonisation for GC-HRMS based non-targeted acquisition for the measurement of chemical exposure agents in human plasma, giving rise to potential for transatlantic monitoring networks.

## 8. Conclusions

Within the PARC initiative, Work Package 4 (WP4) focuses on monitoring and exposure (Marx-Stoelting et al., 2023). Within WP4, Task 4.3 is specifically focused on Innovative methods and tools for monitoring and surveys. This task comprises 67 institutions from Europe and beyond working together so that the further advancing of chemical risk assessment becomes a reality. Four transversal projects are included, respectively focused on concepts and strategies, QA/QC, early warning systems and data processing. Additional real case proof-of-concept projects are planned to implement these outputs in various contexts including human perinatal exposure, human occupational exposure, wastewater-based epidemiology, sentinel animals and environmental monitoring, and chemical food safety areas. Initial steps have outlined core barriers that will be attempted to be overcome in order to advance the implementation of less-conventional sampling strategies and integration of full-scan, high-resolution mass spectrometry and effect-directed analysis into environmental and human monitoring programs for chemical risk assessment.

## 9. Summmary key messages of manuscript

i) From 2022 – 2029, 67 institutions are cooperating within the EU Partnership for Assessment of Risks from Chemicals (PARC) initiative to advance the utility of innovative methods and tools for monitoring and surveys (WP4, Task 4.3) for supporting next-generation chemical risk assessment.

ii) Less conventional sampling, non-targeted mass spectrometry acquisition and effect-directed analysis show promise to advance chemical exposure characterisation but numerous scientific barriers limit their incorporation into regulatory frameworks.

iii) Continuous development of diverse and complementary analytical and computational methodologies is required to improve the detection, annotation, quantification and prioritization of chemicals and/or chemical features presenting current, emerging or future concern.

iv) Uniform reporting practices need to be established and enforced to ensure transparency, reproducibility and comparability of generated results. Mandatory data sharing and metadata reporting, protocol sharing, and quality management procedures should be stipulated. Requirements include establishing fit-for-purpose identification scales, method performance criteria and software quality standards alongside mechanisms to assess compliance.

v) A network of harmonised laboratories operating standardised innovative methods for chemical exposure characterization should be established for use as a tool within a European early warning system for chemical risks.

## Declaration of competing financial interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This research was co-funded from the European Union's Horizon Europe project PARC (101057014). ŽT, HH, JK and EJP acknowledge the research infrastructure RECETOX RI (LM2023069), H2020 CETOCOEN Excellence 857560 and OP RDE CZ.02.1.01/0.0/0.0/17_043/0009632) and CETOCOEN PLUS CZ.02.1.01/0.0/0.0/15_003/0000469). LB acknowledges funding through Research Foundation Flanders (FWO) fellowship (11G1821N). AC acknowledges the FWO research infrastructure EIRENE-Flanders (FWO I001923N) and the Exposome Centre of Excellence of the University of Antwerp (BOF grant, Antigoon database number 41222). This publication reflects only the authors' views, and the European Commission is not responsible for any use that may be made of the information it contains. The authors kindly thank Karl Lilja, Anja Duffek, Christophe Rousselle and Lutz Ahrens for their comments and suggestions during manuscript preparation.

## References

Afgan, E., Nekrutenko, A., Grüning, Bjórn A., Blankenberg, D., Goecks, J., Schatz, M.C., Ostrovsky, A.E., Mahmoud, A., Lonie, A.J., Syme, A., Fouilloux, A., Bretaudeau, A., Nekrutenko, A., Kumar, A., Eschenlauer, A.C., DeSanto, A.D., Guerler, A., Serrano-Solano, B., Batut, B., Grüning, Björn A., Langhorst, B.W., Carr, B., Raubenolt, B.A., Hyde, C.J., Bromhead, C.J., Barnett, C.B., Royaux, C., Gallardo, C., Blankenberg, D., Fornika, D.J., Baker, D., Bouvier, D., Clements, D., de Lima Morais, D.A., Tabernero, D.L., Lariviere, D., Nasr, E., Afgan, E., Zambelli, F., Heyl, F., Psomopoulos, F., Coppens, F., Price, G.R., Cuccuru, G., Corguillé, G. Le, Von Kuster, G., Akbulut, G.G., Rasche, H., Hotz, H.-R., Eguinoa, I., Makunin, I., Ranawaka, I.J., Taylor, J.P., Joshi, J., Hillman-Jackson, J., Goecks, J., Chilton, J.M., Kamali, K., Suderman, K., Poterlowicz, K., Yvan, L.B., Lopez-Delisle, L., Sargent, L., Bassetti, M.E., Tangaro, M.A., van den Beek, M., Čech, M., Bernt, M., Fahrner, M., Tekman, M., Föll, M.C., Schatz, M.C., Crusoe, M.R., Roncoroni, M., Kucher, N., Coraor, N., Stoler, N., Rhodes, N., Soranzo, N., Pinter, N., Goonasekera, N.A., Moreno, P.A., Videm, P., Melanie, P., Mandreoli, P., Jagtap, P.D., Gu, Q., Weber, R.J.M., Lazarus, R., Vorderman, R.H.P., Hiltemann, S., Golitsynskiy, S., Garg, S., Bray, S.A., Gladman, S.L., Leo, S., Mehta, S.P., Griffin, T.J., Jalili, V., Yves, V., Wen, V., Nagampalli, V.K., Bacon, W.A., de Koning, W., Maier, W., Briggs, P.J., 2022. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2022 update. Nucleic Acids Res 50, W345–W351. https://doi.org/10.1093/nar/gkac247

Alseekh, S., Aharoni, A., Brotman, Y., Contrepois, K., D'Auria, J., Ewald, J., C. Ewald, J., Fraser, P.D., Giavalisco, P., Hall, R.D., Heinemann, M., Link, H., Luo, J., Neumann, S., Nielsen, J., Perez de Souza, L., Saito, K., Sauer, U., Schroeder, F.C., Schuster, S., Siuzdak, G., Skirycz, A., Sumner, L.W., Snyder, M.P., Tang, H., Tohge, T., Wang, Y., Wen, W., Wu, S., Xu, G., Zamboni, N., Fernie, A.R., 2021. Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices. Nat Methods 18, 747–756. https://doi.org/10.1038/s41592-021-01197-1

Altenburger, R., Brack, W., Burgess, R.M., Busch, W., Escher, B.I., Focks, A., Mark Hewitt, L., Jacobsen, B.N., de Alda, M.L., Ait-Aissa, S., Backhaus, T., Ginebreda, A., Hilscherová, K., Hollender, J., Hollert, H., Neale, P.A., Schulze, T., Schymanski, E.L., Teodorovic, I., Tindall, A.J., de Aragão Umbuzeiro, G., Vrana, B., Zonja, B., Krauss, M., 2019. Future water quality monitoring: improving the balance between exposure and toxicity assessments of real-world pollutant mixtures. Environ Sci Eur 31, 1–17. https://doi.org/10.1186/s12302-019-0193-1

Alygizakis, N., Lestremau, F., Gago-Ferrero, P., Gil-Solsona, R., Arturi, K., Hollender, J., Schymanski, E.L., Dulio, V., Slobodnik, J., Thomaidis, N.S., 2023. Towards a harmonized identification scoring system in LC-HRMS/MS based non-target screening (NTS) of emerging contaminants. TrAC Trends in Analytical Chemistry 159, 116944. https://doi.org/10.1016/j.trac.2023.116944

Alygizakis, N.A., Samanipour, S., Hollender, J., Ibáñez, M., Kaserzon, S., Kokkali, V., Van Leerdam, J.A., Mueller, J.F., Pijnappels, M., Reid, M.J., Schymanski, E.L., Slobodnik, J., Thomaidis, N.S., Thomas, K. V., 2018. Exploring the Potential of a Global Emerging Contaminant Early Warning Network through the Use of Retrospective Suspect Screening with High-Resolution Mass Spectrometry. Environ Sci Technol 52, 5135–5144. https://doi.org/10.1021/acs.est.8b00365

ANSES, 2020. Draft proposal for a European Partnership under Horizon Europe Partnership for the Assessment of Risk from Chemicals (PARC).

Arturi, K., Hollender, J., 2023. Machine Learning-Based Hazard-Driven Prioritization of Features in Nontarget Screening of Environmental High-Resolution Mass Spectrometry Data. Environ Sci Technol. https://doi.org/10.1021/acs.est.3c00304

Bastian, S., Youngjoon, J., Sarit, K., Amy, H.L., Pradeep, D., Jake, O., Maria Jose, G.R., Sara, G.G., Jochen, M.F., Kevin, T. V., Saer, S., 2020. An assessment of Quality Assurance/Quality Control Efforts in High Resolution Mass Spectrometry Non-Target Workflows for Analysis of Environmental Samples. TrAC Trends in Analytical Chemistry 133, 116063. https://doi.org/10.1016/j.trac.2020.116063

Bettencourt da Silva, R., Ellison, S.L.R., 2021. Eurachem/CITAC Guide: Assessment of performance and uncertainty in qualitative chemical analysis.

Black, G., Lowe, C., Anumol, T., Bade, J., Favela, K., Lai, Y., Knolhoff, A., Mceachran, A., Nuñez, J., Fisher, C., Peter, K., 2022. Exploring chemical space in non - targeted analysis : a proposed ChemSpace tool.

Bopp, S.K., Kienzler, A., Richarz, A.-N., van der Linden, S.C., Paini, A., Parissis, N., Worth, A.P., 2019. Regulatory assessment and risk management of chemical mixtures: challenges and ways forward. Crit Rev Toxicol 0, 1–16. https://doi.org/10.1080/10408444.2019.1579169

Botana, L.M., 2016. Toxicological Perspective on Climate Change: Aquatic Toxins. Chem Res Toxicol 29, 619–625. https://doi.org/10.1021/acs.chemrestox.6b00020

Brack, W., Aissa, S.A., Backhaus, T., Dulio, V., Escher, B.I., Faust, M., Hilscherova, K., Hollender, J., Hollert, H., Müller, C., Munthe, J., Posthuma, L., Seiler, T.B., Slobodnik, J., Teodorovic, I., Tindall, A.J., de Aragão Umbuzeiro, G., Zhang, X., Altenburger, R., 2019. Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality. Environ Sci Eur 31, 4–9. https://doi.org/10.1186/s12302-019-0192-2

Brack, W., Ait-Aissa, S., Burgess, R.M., Busch, W., Creusot, N., Di Paolo, C., Escher, B.I., Mark Hewitt, L., Hilscherova, K., Hollender, J., Hollert, H., Jonker, W., Kool, J., Lamoree, M., Muschket, M., Neumann, S., Rostkowski, P., Ruttkies, C., Schollee, J., Schymanski, E.L., Schulze, T., Seiler, T.B., Tindall, A.J., De Aragão Umbuzeiro, G., Vrana, B., Krauss, M., 2016. Effect-directed analysis supporting monitoring of aquatic environments - An in-depth overview. Science of the Total Environment 544, 1073–1118. https://doi.org/10.1016/j.scitotenv.2015.11.102

Brion, F., De Gussem, V., Buchinger, S., Hollert, H., Carere, M., Porcher, J.M., Piccini, B., Féray, C., Dulio, V., Könemann, S., Simon, E., Werner, I., Kase, R., Aït-Aïssa, S., 2019. Monitoring estrogenic activities of waste and surface waters using a novel in vivo zebrafish embryonic (EASZY) assay: Comparison with in vitro cell-based assays and determination of effect-based trigger values. Environ Int 130, 104896. https://doi.org/10.1016/j.envint.2019.06.006

Buendia, P., Bradley, R.M., Taylor, T.J., Schymanski, E.L., Patti, G.J., Kabuka, M.R., 2019. Ontology-based metabolomics data integration with quality control. Bioanalysis 11, 1139–1154. https://doi.org/10.4155/bio-2018-0303

Burgess, R.M., Ho, K.T., Brack, W., Lamoree, M., 2013. Effects-directed analysis (EDA) and toxicity identification evaluation (TIE): Complementary but different approaches for diagnosing causes of environmental toxicity. Environ Toxicol Chem 32, 1935–1945. https://doi.org/10.1002/etc.2299

Caballero-Casero, N., Belova, L., Vervliet, P., Antignac, J.-P., Castaño, A., Debrauwer, L., López, M.E., Huber, C., Klanova, J., Krauss, M., Lommen, A., Mol, H.G.J., Oberacher, H., Pardo, O., Price, E.J., Reinstadler, V., Vitale, C.M., van Nuijs, A.L.N., Covaci, A., 2021. Towards harmonised criteria in quality assurance and quality control of suspect and non-target LC-HRMS analytical workflows for screening of emerging contaminants in human biomonitoring. TrAC Trends in Analytical Chemistry 136, 116201. https://doi.org/10.1016/j.trac.2021.116201

Celma, A., Sancho, J. V., Schymanski, E.L., Fabregat-Safont, D., Ibáñez, M., Goshawk, J., Barknowitz, G., Hernández, F., Bijlsma, L., 2020. Improving Target and Suspect Screening High-Resolution Mass Spectrometry Workflows in Environmental Analysis by Ion Mobility Separation. Environ Sci Technol 54, 15120–15131. https://doi.org/10.1021/acs.est.0c05713

Chang, H., Colby, S.M., Du, X., Gomez, J.D., Helf, M.J., Kechris, K., Kirkpatrick, C.R., Li, S., Patti, G.J., Renslow, R.S., Subramaniam, S., Verma, M., Xia, J., Young, J.D., 2020. A Practical Guide to Metabolomics Software Development. https://doi.org/10.1021/acs.analchem.0c03581

Charbonnet, J.A., McDonough, C.A., Xiao, F., Schwichtenberg, T., Cao, D., Kaserzon, S., Thomas, K. V., Dewapriya, P., Place, B.J., Schymanski, E.L., Field, J.A., Helbling, D.E., Higgins, C.P., 2022. Communicating Confidence of Per- and Polyfluoroalkyl Substance Identification via High-Resolution Mass Spectrometry. Environ Sci Technol Lett 9, 473–481. https://doi.org/10.1021/acs.estlett.2c00206

Chhaya, R.S., O'Brien, J., Cummins, E., 2022. Feed to fork risk assessment of mycotoxins under climate change influences - recent developments. Trends Food Sci Technol 126, 126–141. https://doi.org/10.1016/j.tifs.2021.07.040

Considine, E.C., Thomas, G., Boulesteix, A.L., Khashan, A.S., Kenny, L.C., 2018. Critical review of reporting of the data analysis step in metabolomics. Metabolomics 14, 7. https://doi.org/10.1007/s11306-017-1299-3

Cousins, I.T., Ng, C.A., Wang, Z., Scheringer, M., 2019. Why is high persistence alone a major cause of concern? Environ Sci Process Impacts 21, 781–792. https://doi.org/10.1039/C8EM00515J

Creusot, N., Casado-Martinez, C., Chiaia-Hernandez, A., Kiefer, K., Ferrari, B.J.D., Fu, Q., Munz, N., Stamm, C., Tlili, A., Hollender, J., 2020. Retrospective screening of high-resolution mass spectrometry archived digital samples can improve environmental risk assessment of emerging contaminants: A case study on antifungal azoles. Environ Int 139, 105708. https://doi.org/10.1016/j.envint.2020.105708

da Silva, R.R., Dorrestein, P.C., Quinn, R.A., 2015. Illuminating the dark matter in metabolomics. Proceedings of the National Academy of Sciences 112, 12549–12550. https://doi.org/10.1073/pnas.1516878112

David, A., Chaker, J., Price, E.J., Bessonneau, V., Chetwynd, A.J., Vitale, C.M., Klánová, J., Walker, D.I., Antignac, J., Barouki, R., Miller, G.W., 2021. Towards a comprehensive characterisation of the human internal chemical exposome: Challenges and perspectives. Environ Int 156, 106630. https://doi.org/10.1016/j.envint.2021.106630

Dévier, M.H., Mazellier, P., Aït-Aïssa, S., Budzinski, H., 2011. New challenges in environmental analytical chemistry: Identification of toxic compounds in complex mixtures. Comptes Rendus Chimie 14, 766–779. https://doi.org/10.1016/j.crci.2011.04.006

Drakvik, E., Altenburger, R., Aoki, Y., Backhaus, T., Bahadori, T., Barouki, R., Brack, W., Cronin, M.T.D., Demeneix, B., Hougaard Bennekou, S., van Klaveren, J., Kneuer, C., Kolossa-Gehring, M., Lebret, E., Posthuma, L., Reiber, L., Rider, C., Rüegg, J., Testa, G., van der Burg, B., van der Voet, H., Warhurst, A.M., van de Water, B., Yamazaki, K., Öberg, M., Bergman, Å., 2020. Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. Environ Int 134. https://doi.org/10.1016/j.envint.2019.105267

Egeghy, P.P., Judson, R., Gangwal, S., Mosher, S., Smith, D., Vail, J., Cohen Hubal, E.A., 2012. The exposure data landscape for manufactured chemicals. Science of the Total Environment 414, 159–166. https://doi.org/10.1016/j.scitotenv.2011.10.046

EMCDDA, 2019. Operating guidelines for the European Union Early Warning System on new psychoactive substances 2006, 1–27.

Engels, H.-W., Weidenhaupt, H.-J., Abele, M., Pieroth, M., Hofmann, W., 2000. Rubber, 4. Chemicals and Additives. Ullmann's Encyclopedia of Industrial Chemistry. https://doi.org/10.1002/14356007.a23_365

Escher, B.I., Fenner, K., 2011. Recent advances in environmental risk assessment of transformation products. Environ Sci Technol 45, 3835–3847. https://doi.org/10.1021/es1030799

Escher, B.I., Lamoree, M., Antignac, J., Scholze, M., Herzler, M., Hamers, T., Jensen, T.K., Audebert, M., Busquet, F., Maier, D., Oelgeschläger, M., Jo, M., Boye, H., Schmeisser, S., Dervilly, G., Piumatti, M., König, M., Renko, K., Margalef, M., Cariou, R., Ma, Y., Treschow, A.F., Kortenkamp, A., Vinggaard, A.M., 2022. Mixture Risk Assessment of Complex Real-Life Mixtures — The PANORAMIX Project.

Escher, B.I., Stapleton, H.M., Schymanski, E.L., 2020. Tracking complex mixtures of chemicals in our changing environment. Science (1979) 367, 388–392. https://doi.org/10.1126/science.aay6636

European Agency for Safety and Health at Work (EU-OSHA), 2017. Methodologies to identify work-related diseases: Review on sentinel and alert systems. https://doi.org/10.2802/053155

European Commission, 2023a. COMMISSION REGULATION (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006.

European Commission, 2023b. of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

European Commission, 2022. European Commission, Safe and sustainable by design chemicals and materials: a European assessment framework. Publications Office of the European Union.

European Commission, 2021. COMMISSION IMPLEMENTING REGULATION (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to 48–119.

European Commission, 2018a. COMMISSION IMPLEMENTING DECISION (EU) 2022/1307 of 22 July 2022 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council 2016, 48–119.

European Commission, 2018b. COMMISSION IMPLEMENTING DECISION (EU) 2019/417 of 8 November 2018 laying down guidelines for the management of the European Union Rapid Information System 'RAPEX' established under Article 12 of Directive 2001/95/EC on general product safety and its notification system.

European Commission, 2012. The combination effects of chemicals Chemical mixtures.

European Commission, 2008a. DIRECTIVE 2008/50/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 May 2008 on ambient air quality and cleaner air for Europe OJ L 152.

European Commission, 2008b. DIRECTIVE 2008/56/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive) OJ L 164, 19.

European Commission, 2006. DIRECTIVE 2006/118/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the protection of 12 December 2006 of groundwater against pollution deterioration OJL364, 5.

European Commission, 2002. REGULATION (EC) No 178/2002 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCILof 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

European Commission, 2000. DIRECTIVE 2000/60/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 October 2000 establishing a framework for Community action in the field of water policy OJ L 327, 1.

European Human Biomonitoring Initiative, 2022. The Human Biomonitoring Laboratory Network, a key asset in supporting human biomonitoring and risk assessment in Europe. HBM4EU Newspaper 23–28.

Fisher, C.M., Peter, K.T., Newton, S.R., Schaub, A.J., Sobus, J.R., 2022. Approaches for assessing performance of high-resolution mass spectrometry–based non-targeted analysis methods. Anal Bioanal Chem 414, 6455–6471. https://doi.org/10.1007/s00216-022-04203-3

Fu, Q., Meyer, C., Patrick, M., Kosfeld, V., Rüdel, H., Koschorreck, J., Hollender, J., 2022. Comprehensive screening of polar emerging organic contaminants including PFASs and evaluation of the trophic transfer behavior in a freshwater food web. Water Res 218. https://doi.org/10.1016/j.watres.2022.118514

Gago-Ferrero, P., Bletsou, A.A., Damalas, D.E., Aalizadeh, R., Alygizakis, N.A., Singer, H.P., Hollender, J., Thomaidis, N.S., 2020. Wide-scope target screening of >2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes. J Hazard Mater 387, 121712. https://doi.org/10.1016/j.jhazmat.2019.121712

Ganzleben, C., Antignac, J.P., Barouki, R., Castaño, A., Fiddicke, U., Klánová, J., Lebret, E., Olea, N., Sarigiannis, D., Schoeters, G.R., Sepai, O., Tolonen, H., Kolossa-Gehring, M., 2017. Human biomonitoring as a tool to support chemicals regulation in the European Union. Int J Hyg Environ Health 220, 94–97. https://doi.org/10.1016/j.ijheh.2017.01.007

García-Reyes, J.F., Hernando, M.D., Ferrer, C., Molina-Díaz, A., Fernández-Alba, A.R., 2007. Large Scale Pesticide Multiresidue Methods in Food Combining Liquid Chromatography– Time-of-Flight Mass Spectrometry and Tandem Mass Spectrometry. Anal Chem 79, 7308–7323. https://doi.org/10.1021/ac070855v

Gauglitz, J.M., West, K.A., Bittremieux, W., Williams, C.L., Weldon, K.C., Panitchpakdi, M., Di Ottavio, F., Aceves, C.M., Brown, E., Sikora, N.C., Jarmusch, A.K., Martino, C., Tripathi, A., Meehan, M.J., Dorrestein, K., Shaffer, J.P., Coras, R., Vargas, F., Goldasich, L.D., Schwartz, T., Bryant, M., Humphrey, G., Johnson, A.J., Spengler, K., Belda-Ferre, P., Diaz, E., McDonald, D., Zhu, Q., Elijah, E.O., Wang, M., Marotz, C., Sprecher, K.E., Vargas-Robles, D., Withrow, D., Ackermann, G., Herrera, L., Bradford, B.J., Marques, L.M.M., Amaral, J.G., Silva, R.M., Veras, F.P., Cunha, T.M., Oliveira, R.D.R., Louzada-Junior, P., Mills, R.H., Piotrowski, P.K., Servetas, S.L., Da Silva, S.M., Jones, C.M., Lin, N.J., Lippa, K.A., Jackson, S.A., Daouk, R.K., Galasko, D., Dulai, P.S., Kalashnikova, T.I., Wittenberg, C., Terkeltaub, R., Doty, M.M., Kim, J.H., Rhee, K.E., Beauchamp-Walters, J., Wright, K.P., Dominguez-Bello, M.G., Manary, M., Oliveira, M.F., Boland, B.S., Lopes, N.P., Guma, M., Swafford, A.D., Dutton, R.J., Knight, R., Dorrestein, P.C., 2022. Enhancing untargeted metabolomics using metadata-based source annotation. Nat Biotechnol 40, 1774–1779. https://doi.org/10.1038/s41587-022-01368-1

Grulke, C.M., Williams, A.J., Thillanadarajah, I., Richard, A.M., 2019. EPA's DSSTox database: History of development of a curated chemistry resource supporting computational toxicology research. Computational Toxicology 12, 100096. https://doi.org/10.1016/j.comtox.2019.100096

Gustavsson, M., Molander, S., Backhaus, T., Kristiansson, E., 2023. Risk assessment of chemicals and their mixtures are hindered by scarcity and inconsistencies between different environmental exposure limits. Environ Res 225, 115372. https://doi.org/10.1016/j.envres.2023.115372

Haug, K., Cochrane, K., Nainala, V.C., Williams, M., Chang, J., Jayaseelan, K.V., O'Donovan, C., 2020. MetaboLights: a resource evolving in response to the needs of its scientific community. Nucleic Acids Res 48, D440–D444. https://doi.org/10.1093/nar/gkz1019

Hazardous Substances Advisory Committee, 2021. Recommendations for a Prioritisation and Early Warning System ( PEWS ) on Chemicals in the Environment 1–15.

Heys, K.A., Shore, R.F., Pereira, M.G., Jones, K.C., Martin, F.L., 2016. Risk assessment of environmental mixture effects. RSC Adv 6, 47844–47857. https://doi.org/10.1039/c6ra05406d

Hollender, J., Schymanski, E.L., Singer, H.P., Ferguson, P.L., 2017. Nontarget Screening with High Resolution Mass Spectrometry in the Environment: Ready to Go? Environ Sci Technol 51, 11505–11512. https://doi.org/10.1021/acs.est.7b02184

Hollender, J., van Bavel, B., Dulio, V., Farmen, E., Furtmann, K., Koschorreck, J., Kunkel, U., Krauss, M., Munthe, J., Schlabach, M., Slobodnik, J., Stroomberg, G., Ternes, T., Thomaidis, N.S., Togola, A., Tornero, V., 2019. High resolution mass spectrometry-based non-target screening can support regulatory environmental monitoring and chemicals management. Environ Sci Eur 31, 42. https://doi.org/10.1186/s12302-019-0225-x

Huber, C., Nijssen, R., Mol, H., Philippe Antignac, J., Krauss, M., Brack, W., Wagner, K., Debrauwer, L., Maria Vitale, C., James Price, E., Klanova, J., Garlito Molina, B., Leon, N., Pardo, O., Fernández, S.F., Szigeti, T., Középesy, S., Šulc, L., Čupr, P., Mārtiņsone, I., Akülova, L., Ottenbros, I., Vermeulen, R., Vlaanderen, J., Luijten, M., Lommen, A., 2022. A large scale multi-laboratory suspect screening of pesticide metabolites in human biomonitoring: From tentative annotations to verified occurrences. Environ Int 168, 107452. https://doi.org/10.1016/j.envint.2022.107452

Jarmusch, A.K., Wang, M., Aceves, C.M., Advani, R.S., Aguirre, S., Aksenov, A.A., Aleti, G., Aron, A.T., Bauermeister, A., Bolleddu, S., Bouslimani, A., Caraballo Rodriguez, A.M., Chaar, R., Coras, R., Elijah, E.O., Ernst, M., Gauglitz, J.M., Gentry, E.C., Husband, M., Jarmusch, S.A., Jones, K.L., Kamenik, Z., Le Gouellec, A., Lu, A., McCall, L.I., McPhail, K.L., Meehan, M.J., Melnik, A. V., Menezes, R.C., Montoya Giraldo, Y.A., Nguyen, N.H., Nothias, L.F., Nothias-Esposito, M., Panitchpakdi, M., Petras, D., Quinn, R.A., Sikora, N., van der Hooft, J.J.J., Vargas, F., Vrbanac, A., Weldon, K.C., Knight, R., Bandeira, N., Dorrestein, P.C., 2020. ReDU: a framework to find and reanalyze public mass spectrometry data. Nat Methods 17, 901–904. https://doi.org/10.1038/s41592-020-0916-7

Jeon, J., Kurth, D., Hollender, J., 2013. Biotransformation Pathways of Biocides and Pharmaceuticals in Freshwater Crustaceans Based on Structure Elucidation of Metabolites Using High Resolution Mass Spectrometry. Chem Res Toxicol 26, 313–324. https://doi.org/10.1021/tx300457f

Johnson, A.C., Jin, X., Nakada, N., Sumpter, J.P., 2020. Learning from the past and considering the Future of Chemicals in the Environment. Science (1979) 367, 384–387.

Jonkers, T.J.H., Meijer, J., Vlaanderen, J.J., Vermeulen, R.C.H., Houtman, C.J., Hamers, T., Lamoree, M.H., 2022. High-Performance Data Processing Workflow Incorporating Effect-Directed Analysis for Feature Prioritization in Suspect and Nontarget Screening. Environ Sci Technol 56, 1639–1651. https://doi.org/10.1021/acs.est.1c04168

Khanna, V., Ranganathan, S., 2009. Physiochemical property space distribution among human metabolites, drugs and toxins. BMC Bioinformatics 10, 1–18. https://doi.org/10.1186/1471-2105-10-S15-S10

Kirwan, J.A., Gika, H., Beger, R.D., Bearden, D., Dunn, W.B., Goodacre, R., Theodoridis, G., Witting, M., Yu, L.R., Wilson, I.D., 2022. Quality assurance and quality control reporting in untargeted

metabolic phenotyping: mQACC recommendations for analytical quality management. Metabolomics 18, 1–16. https://doi.org/10.1007/s11306-022-01926-3

Knolhoff, A.M., Premo, J.H., Fisher, C.M., 2021. A proposed quality control standard mixture and its uses for evaluating nontargeted and suspect screening LC/HR-MS method performance. Anal Chem 93, 1596–1603. https://doi.org/10.1021/acs.analchem.0c04036

Kodra, D., Pousinis, P., Vorkas, P.A., Kademoglou, K., Liapikos, T., Pechlivanis, A., Virgiliou, C., Wilson, I.D., Gika, H., Theodoridis, G., 2022. Is Current Practice Adhering to Guidelines Proposed for Metabolite Identification in LC-MS Untargeted Metabolomics? A Meta-Analysis of the Literature. J Proteome Res 21, 590–598. https://doi.org/10.1021/acs.jproteome.1c00841

Koelmel, J.P., Xie, H., Price, E.J., Lin, E.Z., Manz, K.E., Stelben, P., Paige, M.K., Papazian, S., Okeme, J., Jones, D.P., Barupal, D., Bowden, J.A., Rostkowski, P., Pennell, K.D., Nikiforov, V., Wang, T., Hu, X., Lai, Y., Miller, G.W., Walker, D.I., Martin, J.W., Godri Pollitt, K.J., 2022. An actionable annotation scoring framework for gas chromatography-high-resolution mass spectrometry. Exposome 2. https://doi.org/10.1093/exposome/osac007

Kortenkamp, A., Faust, M., 2018. Regulate to reduce chemical mixture risk. Science (1979) 361, 224–226. https://doi.org/10.1126/science.aat9219

Krauss, M., Singer, H., Hollender, J., 2010. LC-high resolution MS in environmental analysis: From target screening to the identification of unknowns. Anal Bioanal Chem 397, 943–951. https://doi.org/10.1007/s00216-010-3608-9

Kruve, A., Kiefer, K., Hollender, J., 2021. Benchmarking of the quantification approaches for the non-targeted screening of micropollutants and their transformation products in groundwater. Anal Bioanal Chem 413, 1549–1559. https://doi.org/10.1007/s00216-020-03109-2

Lai, A., Clark, A.M., Escher, B.I., Fernandez, M., Mcewen, L.R., Tian, Z., Wang, Z., Schymanski, E.L., 2022. The Next Frontier of Environmental Unknowns: Substances of Unknown or Variable Composition, Complex Reaction Products, or Biological Materials (UVCBs). Environ Sci Technol 56, 7448–7466. https://doi.org/10.1021/acs.est.2c00321

Landrigan, P.J., Fuller, R., Acosta, N.J.R., Adeyi, O., Arnold, R., Basu, N. (Nil), Baldé, A.B., Bertollini, R., Bose-O'Reilly, S., Boufford, J.I., Breysse, P.N., Chiles, T., Mahidol, C., Coll-Seck, A.M., Cropper, M.L., Fobil, J., Fuster, V., Greenstone, M., Haines, A., Hanrahan, D., Hunter, D., Khare, M., Krupnick, A., Lanphear, B., Lohani, B., Martin, K., Mathiasen, K. V., McTeer, M.A., Murray, C.J.L., Ndahimananjara, J.D., Perera, F., Potočnik, J., Preker, A.S., Ramesh, J., Rockström, J., Salinas, C., Samson, L.D., Sandilya, K., Sly, P.D., Smith, K.R., Steiner, A., Stewart, R.B., Suk, W.A., van Schayck, O.C.P., Yadama, G.N., Yumkella, K., Zhong, M., 2018. The Lancet Commission on pollution and health. The Lancet 391, 462–512. https://doi.org/10.1016/S0140-6736(17)32345-0

Li, Yong, Ruan, Q., Li, Yanli, Ye, G., Lu, X., Lin, X., Xu, G., 2012. A novel approach to transforming a non-targeted metabolic profiling method to a pseudo-targeted method using the retention time locking gas chromatography/mass spectrometry-selected ions monitoring. J Chromatogr A 1255, 228–236. https://doi.org/10.1016/j.chroma.2012.01.076

Liigand, P., Liigand, J., Cuyckens, F., Vreeken, R.J., Kruve, A., 2018. Ionisation efficiencies can be predicted in complicated biological matrices: A proof of concept. Anal Chim Acta 1032, 68–74. https://doi.org/10.1016/j.aca.2018.05.072

Liigand, P., Liigand, J., Kaupmees, K., Kruve, A., 2021. 30 Years of research on ESI/MS response: Trends, contradictions and applications. Anal Chim Acta 1152. https://doi.org/10.1016/j.aca.2020.11.049

Marx-Stoelting, P., Rivière, G., Luijten, M., Aiello-Holden, K., Bandow, N., Baken, K., Cañas, A., Castano, A., Denys, S., Fillol, C., Herzler, M., Iavicoli, I., Karakitsios, S., Klanova, J., Kolossa-Gehring, M., Koutsodimou, A., Vicente, J.L., Lynch, I., Namorado, S., Norager, S., Pittman, A., Rotter, S., Sarigiannis, D., Silva, M.J., Theunis, J., Tralau, T., Uhl, M., van Klaveren, J., Wendt-Rasch, L., Westerholm, E., Rousselle, C., Sanders, P., 2023. A walk in the PARC: developing and implementing 21st century chemical risk assessment in Europe. Arch Toxicol 97, 893–908. https://doi.org/10.1007/s00204-022-03435-7

Mccord, J.P., Groff, L.C., Sobus, J.R., 2022a. Quantitative non-targeted analysis : Bridging the gap between contaminant discovery and risk characterization. Environ Int 158, 107011. https://doi.org/10.1016/j.envint.2021.107011

Mccord, J.P., Groff, L.C., Sobus, J.R., 2022b. Quantitative non-targeted analysis : Bridging the gap between contaminant discovery and risk characterization. Environ Int 158, 107011. https://doi.org/10.1016/j.envint.2021.107011

Meijer, J., Lamoree, M., Hamers, T., Antignac, J.P., Hutinet, S., Debrauwer, L., Covaci, A., Huber, C., Krauss, M., Walker, D.I., Schymanski, E.L., Vermeulen, R., Vlaanderen, J., 2021. An annotation database for chemicals of emerging concern in exposome research. Environ Int 152. https://doi.org/10.1016/j.envint.2021.106511

Mijangos, L., Krauss, M., De Miguel, L., Ziarrusta, H., Olivares, M., Zuloaga, O., Izagirre, U., Schulze, T., Brack, W., Prieto, A., Etxebarria, N., 2020. Application of the Sea Urchin Embryo Test in Toxicity Evaluation and Effect-Directed Analysis of Wastewater Treatment Plant Effluents. Environ Sci Technol 54, 8890–8899. https://doi.org/10.1021/acs.est.0c01504

MohammadiPeyhani, H., Hafner, J., Sveshnikova, A., Viterbo, V., Hatzimanikatis, V., 2022. Expanding biochemical knowledge and illuminating metabolic dark matter with ATLASx. Nat Commun 13, 1560. https://doi.org/10.1038/s41467-022-29238-z

Mohammed Taha, H., Aalizadeh, R., Alygizakis, N., Antignac, J.P., Arp, H.P.H., Bade, R., Baker, N., Belova, L., Bijlsma, L., Bolton, E.E., Brack, W., Celma, A., Chen, W.L., Cheng, T., Chirsir, P., Čirka, Ľ., D'Agostino, L.A., Djoumbou Feunang, Y., Dulio, V., Fischer, S., Gago-Ferrero, P., Galani, A., Geueke, B., Głowacka, N., Glüge, J., Groh, K., Grosse, S., Haglund, P., Hakkinen, P.J., Hale, S.E., Hernandez, F., Janssen, E.M.L., Jonkers, T., Kiefer, K., Kirchner, M., Koschorreck, J., Krauss, M., Krier, J., Lamoree, M.H., Letzel, M., Letzel, T., Li, Q., Little, J., Liu, Y., Lunderberg, D.M., Martin, J.W., McEachran, A.D., McLean, J.A., Meier, C., Meijer, J., Menger, F., Merino, C., Muncke, J., Muschket, M., Neumann, M., Neveu, V., Ng, K., Oberacher, H., O'Brien, J., Oswald, P., Oswaldova, M., Picache, J.A., Postigo, C., Ramirez, N., Reemtsma, T., Renaud, J., Rostkowski, P., Rüdel, H., Salek, R.M., Samanipour, S., Scheringer, M., Schliebner, I., Schulz, W., Schulze, T., Sengl, M., Shoemaker, B.A., Sims, K., Singer, H., Singh, R.R., Sumarah, M., Thiessen, P.A., Thomas, K. V., Torres, S., Trier, X., van Wezel, A.P., Vermeulen, R.C.H., Vlaanderen, J.J., von der Ohe, P.C., Wang, Z., Williams, A.J., Willighagen, E.L., Wishart, D.S., Zhang, J., Thomaidis, N.S., Hollender, J., Slobodnik, J., Schymanski, E.L., 2022. The NORMAN Suspect List Exchange (NORMAN-SLE): facilitating European and worldwide collaboration on suspect screening in high resolution mass spectrometry. Environ Sci Eur 34. https://doi.org/10.1186/s12302-022-00680-6

Monteiro, J., Chaker, J., Martail, A., Moreira, J.C., David, A., Bot, B. Le, 2021. Improving Exposure Assessment Using Non-Targeted and Suspect Screening : The ISO / IEC 17025 : 2017 Quality Standard as a Guideline 1–15.

More, S.J., Hardy, A., Bampidis, V., Benford, D., Hougaard Bennekou, S., Bragard, C., Boesten, J., Halldorsson, T.I., Hernández-Jerez, A.F., Jeger, M.J., Knutsen, H.K., Koutsoumanis, K.P., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J.R., Silano, V., Nielsen, S.S., Schrenk, D., Solecki, R., Turck, D., Younes, M., Benfenati, E., Castle, L., Cedergreen, N., Laskowski, R., Leblanc, J.C., Kortenkamp, A., Ragas, A., Posthuma, L., Svendsen, C., Testai, E., Dujardin, B., Kass, G.E.N., Manini, P., Zare Jeddi, M., Dorne, J.L.C., Hogstrand, C., 2019. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 17. https://doi.org/10.2903/j.efsa.2019.5634

Murray, C.J.L., Aravkin, A.Y., Zheng, P., Abbafati, C., Abbas, K.M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abegaz, K.H., Abolhassani, H., Aboyans, V., Abreu, L.G., Abrigo, M.R.M., Abualhasan, A., Abu-Raddad, L.J., Abushouk, A.I., Adabi, M., Adekanmbi, V., Adeoye, A.M., Adetokunboh, O.O., Adham, D., Advani, S.M., Agarwal, G., Aghamir, S.M.K., Agrawal, A., Ahmad, T., Ahmadi, K., Ahmadi, M., Ahmadieh, H., Ahmed, M.B., Akalu, T.Y., Akinyemi, R.O., Akinyemiju, T., Akombi, B., Akunna, C.J., Alahdab, F., Al-Aly, Z., Alam, K., Alam, S., Alam, T., Alanezi, F.M., Alanzi, T.M., Alemu, B. wassihun, Alhabib, K.F., Ali, M., Ali, S., Alicandro, G., Alinia, C., Alipour, V., Alizade, H., Aljunid, S.M., Alla, F., Allebeck, P., Almasi-Hashiani, A., Al-Mekhlafi, H.M., Alonso, J., Altirkawi, K.A., Amini-Rarani, M., Amiri, F., Amugsi, D.A., Ancuceanu, R., Anderlini, D., Anderson, J.A., Andrei, C.L., Andrei, T., Angus, C., Anjomshoa, M., Ansari, F., Ansari-Moghaddam, A., Antonazzo, I.C., Antonio, C.A.T., Antony, C.M., Antriyandarti, E., Anvari, D., Anwer, R., Appiah, S.C.Y., Arabloo, J., Arab-Zozani, M., Ariani, F., Armoon, B., Ärnlöv, J., Arzani, A., Asadi-Aliabadi, M., Asadi-Pooya, A.A., Ashbaugh, C., Assmus, M., Atafar, Z., Atnafu, D.D., Atout, M.M. d. W., Ausloos, F., Ausloos, M., Ayala Quintanilla, B.P., Ayano, G., Ayanore, M.A., Azari, S., Azarian, G., Azene, Z.N., Badawi, A., Badiye, A.D., Bahrami, M.A., Bakhshaei, M.H., Bakhtiari, A., Bakkannavar, S.M., Baldasseroni, A., Ball, K., Ballew, S.H., Balzi, D., Banach, M., Banerjee, S.K., Bante, A.B., Baraki, A.G., Barker-Collo, S.L., Bärnighausen, T.W., Barrero, L.H., Barthelemy, C.M., Barua, L., Basu, S., Baune, B.T., Bayati, M., Becker, J.S., Bedi, N., Beghi, E., Béjot, Y., Bell, M.L., Bennitt, F.B., Bensenor, I.M., Berhe, K., Berman, A.E., Bhagavathula, A.S., Bhageerathy, R., Bhala, N., Bhandari, D., Bhattacharyya, K., Bhutta, Z.A., Bijani, A., Bikbov, B., Bin Sayeed, M.S., Biondi, A., Birihane, B.M., Bisignano, C., Biswas, R.K., Bitew, H., Bohlouli, S., Bohluli, M., Boon-Dooley, A.S., Borges, G., Borzì, A.M., Borzouei, S., Bosetti, C., Boufous, S., Braithwaite, D., Breitborde, N.J.K., Breitner, S., Brenner, H., Briant, P.S., Briko, A.N., Briko, N.I., Britton, G.B., Bryazka, D., Bumgarner, B.R., Burkart, K., Burnett, R.T., Burugina Nagaraja, S., Butt, Z.A., Caetano dos Santos, F.L., Cahill, L.E., Cámera, L.L.A., Campos-Nonato, I.R., Cárdenas, R., Carreras, G., Carrero, J.J., Carvalho, F., Castaldelli-Maia, J.M., Castañeda-Orjuela, C.A., Castelpietra, G., Castro, F., Causey, K., Cederroth, C.R., Cercy, K.M., Cerin, E., Chandan, J.S., Chang, K.L., Charlson, F.J., Chattu, V.K., Chaturvedi, S., Cherbuin, N., Chimed-Ochir, O., Cho, D.Y., Choi, J.Y.J., Christensen, H., Chu, D.T., Chung, M.T., Chung, S.C., Cicuttini, F.M., Ciobanu, L.G., Cirillo, M., Classen, T.K.D., Cohen, A.J., Compton, K., Cooper, O.R., Costa, V.M., Cousin, E., Cowden, R.G., Cross, D.H., Cruz, J.A., Dahlawi, S.M.A., Damasceno, A.A.M., Damiani, G., Dandona, L., Dandona, R., Dangel, W.J., Danielsson, A.K., Dargan, P.I., Darwesh, A.M., Daryani, A., Das, J.K., Das Gupta, Rajat, das Neves, J., Dávila-Cervantes, C.A., Davitoiu, D.V., De Leo, D., Degenhardt, L., DeLang, M., Dellavalle, R.P., Demeke, F.M., Demoz, G.T., Demsie, D.G., Denova-Gutiérrez, E., Dervenis, N., Dhungana, G.P.,

Dianatinasab, M., Dias da Silva, D., Diaz, D., Dibaji Forooshani, Z.S., Djalalinia, S., Do, H.T., Dokova, K., Dorostkar, F., Doshmangir, L., Driscoll, T.R., Duncan, B.B., Duraes, A.R., Eagan, A.W., Edvardsson, D., El Nahas, N., El Sayed, I., El Tantawi, M., Elbarazi, I., Elgendy, I.Y., El-Jaafary, S.I., Elyazar, I.R., Emmons-Bell, S., Erskine, H.E., Eskandarieh, S., Esmaeilnejad, S., Esteghamati, A., Estep, K., Etemadi, A., Etisso, A.E., Fanzo, J., Farahmand, M., Fareed, M., Faridnia, R., Farioli, A., Faro, A., Faruque, M., Farzadfar, F., Fattahi, N., Fazlzadeh, M., Feigin, V.L., Feldman, R., Fereshtehnejad, S.M., Fernandes, E., Ferrara, G., Ferrari, A.J., Ferreira, M.L., Filip, I., Fischer, F., Fisher, J.L., Flor, L.S., Foigt, N.A., Folayan, M.O., Fomenkov, A.A., Force, L.M., Foroutan, M., Franklin, R.C., Freitas, M., Fu, W., Fukumoto, T., Furtado, J.M., Gad, M.M., Gakidou, E., Gallus, S., Garcia-Basteiro, A.L., Gardner, W.M., Geberemariyam, B.S., Gebreslassie, A.A.A.A., Geremew, A., Gershberg Hayoon, A., Gething, P.W., Ghadimi, M., Ghadiri, K., Ghaffarifar, F., Ghafourifard, M., Ghamari, F., Ghashghaee, A., Ghiasvand, H., Ghith, N., Gholamian, A., Ghosh, R., Gill, P.S., Ginindza, T.G.G., Giussani, G., Gnedovskaya, E. V., Goharinezhad, S., Gopalani, S.V., Gorini, G., Goudarzi, H., Goulart, A.C., Greaves, F., Grivna, M., Grosso, G., Gubari, M.I.M., Gugnani, H.C., Guimarães, R.A., Guled, R.A., Guo, G., Guo, Y., Gupta, Rajeev, Gupta, T., Haddock, B., Hafezi-Nejad, N., Hafiz, A., Haj-Mirzaian, Arvin, Haj-Mirzaian, Arya, Hall, B.J., Halvaei, I., Hamadeh, R.R., Hamidi, S., Hammer, M.S., Hankey, G.J., Haririan, H., Haro, J.M., Hasaballah, A.I., Hasan, M.M., Hasanpoor, E., Hashi, A., Hassanipour, S., Hassankhani, H., Havmoeller, R.J., Hay, S.I., Hayat, K., Heidari, G., Heidari-Soureshjani, R., Henrikson, H.J., Herbert, M.E., Herteliu, C., Heydarpour, F., Hird, T.R., Hoek, H.W., Holla, R., Hoogar, P., Hosgood, H.D., Hossain, N., Hosseini, M., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Househ, M., Hsairi, M., Hsieh, V.C. rong, Hu, G., Hu, K., Huda, T.M., Humayun, A., Huynh, C.K., Hwang, B.F., Iannucci, V.C., Ibitoye, S.E., Ikeda, N., Ikuta, K.S., Ilesanmi, O.S., Ilic, I.M., Ilic, M.D., Inbaraj, L.R., Ippolito, H., Iqbal, U., Irvani, S.S.N., Irvine, C.M.S., Islam, M.M., Islam, S.M.S., Iso, H., Ivers, R.Q., Iwu, C.C.D., Iwu, C.J., Iyamu, I.O., Jaafari, J., Jacobsen, K.H., Jafari, H., Jafarinia, M., Jahani, M.A., Jakovljevic, M., Jalilian, F., James, S.L., Janjani, H., Javaheri, T., Javidnia, J., Jeemon, P., Jenabi, E., Jha, R.P., Jha, V., Ji, J.S., Johansson, L., John, O., John-Akinola, Y.O., Johnson, C.O., Jonas, J.B., Joukar, F., Jozwiak, J.J., Jürisson, M., Kabir, A., Kabir, Z., Kalani, H., Kalani, R., Kalankesh, L.R., Kalhor, R., Kanchan, T., Kapoor, N., Karami Matin, B., Karch, A., Karim, M.A., Kassa, G.M., Katikireddi, S.V., Kayode, G.A., Kazemi Karyani, A., Keiyoro, P.N., Keller, C., Kemmer, L., Kendrick, P.J., Khalid, N., Khammarnia, M., Khan, E.A., Khan, M., Khatab, K., Khater, M.M., Khatib, M.N., Khayamzadeh, M., Khazaei, S., Kieling, C., Kim, Y.J., Kimokoti, R.W., Kisa, A., Kisa, S., Kivimäki, M., Knibbs, L.D., Knudsen, A.K.S., Kocarnik, J.M., Kochhar, S., Kopec, J.A., Korshunov, V.A., Koul, P.A., Koyanagi, A., Kraemer, M.U.G., Krishan, K., Krohn, K.J., Kromhout, H., Kuate Defo, B., Kumar, G.A., Kumar, V., Kurmi, O.P., Kusuma, D., La Vecchia, C., Lacey, B., Lal, D.K., Lalloo, R., Lallukka, T., Lami, F.H., Landires, I., Lang, J.J., Langan, S.M., Larsson, A.O., Lasrado, S., Lauriola, P., Lazarus, J. V., Lee, P.H., Lee, S.W.H., LeGrand, K.E., Leigh, J., Leonardi, M., Lescinsky, H., Leung, J., Levi, M., Li, S., Lim, L.L., Linn, S., Liu, Shiwei, Liu, Simin, Liu, Y., Lo, J., Lopez, A.D., Lopez, J.C.F., Lopukhov, P.D., Lorkowski, S., Lotufo, P.A., Lu, A., Lugo, A., Maddison, E.R., Mahasha, P.W., Mahdavi, M.M., Mahmoudi, M., Majeed, A., Maleki, A., Maleki, S., Malekzadeh, R., Malta, D.C., Mamun, A.A., Manda, A.L., Manguerra, H., Mansour-Ghanaei, F., Mansouri, B., Mansournia, M.A., Mantilla Herrera, A.M., Maravilla, J.C., Marks, A., Martin, R. V., Martini, S., Martins-Melo, F.R., Masaka, A., Masoumi, S.Z., Mathur, M.R., Matsushita, K., Maulik, P.K., McAlinden, C., McGrath, J.J., McKee, M., Mehndiratta, M.M., Mehri, F., Mehta, K.M., Memish, Z.A., Mendoza, W., Menezes, R.G., Mengesha, E.W., Mereke, A., Mereta, S.T., Meretoja, A., Meretoja, T.J., Mestrovic, T., Miazgowski, B., Miazgowski, T., Michalek, I.M., Miller, T.R., Mills, E.J., Mini, G.K., Miri, M., Mirica, A., Mirrakhimov, E.M., Mirzaei, H., Mirzaei, M., Mirzaei, R., Mirzaei-Alavijeh, M., Misganaw, A.T., Mithra, P., Moazen, B., Mohammad, D.K.,

Mohammad, Y., Mohammad Gholi Mezerji, N., Mohammadian-Hafshejani, A., Mohammadifard, N., Mohammadpourhodki, R., Mohammed, A.S., Mohammed, H., Mohammed, J.A., Mohammed, S., Mokdad, A.H., Molokhia, M., Monasta, L., Mooney, M.D., Moradi, G., Moradi, M., Moradi-Lakeh, M., Moradzadeh, R., Moraga, P., Morawska, L., Morgado-da-Costa, J., Morrison, S.D., Mosapour, A., Mosser, J.F., Mouodi, S., Mousavi, S.M., Mousavi Khaneghah, A., Mueller, U.O., Mukhopadhyay, S., Mullany, E.C., Musa, K.I., Muthupandian, S., Nabhan, A.F., Naderi, M., Nagarajan, A.J., Nagel, G., Naghavi, M., Naghshtabrizi, B., Naimzada, M.D., Najafi, F., Nangia, V., Nansseu, J.R., Naserbakht, M., Nayak, V.C., Negoi, I., Ngunjiri, J.W., Nguyen, C.T., Nguyen, H.L.T., Nguyen, M., Nigatu, Y.T., Nikbakhsh, R., Nixon, M.R., Nnaji, C.A., Nomura, S., Norrving, B., Noubiap, J.J., Nowak, C., Nunez-Samudio, V., Oţoiu, A., Oancea, B., Odell, C.M., Ogbo, F.A., Oh, I.H., Okunga, E.W., Oladnabi, M., Olagunju, A.T., Olusanya, B.O., Olusanya, J.O., Omer, M.O., Ong, K.L., Onwujekwe, O.E., Orpana, H.M., Ortiz, A., Osarenotor, O., Osei, F.B., Ostroff, S.M., Otstavnov, N., Otstavnov, S.S., Øverland, S., Owolabi, M.O., P A, M., Padubidri, J.R., Palladino, R., Panda-Jonas, S., Pandey, A., Parry, C.D.H., Pasovic, M., Pasupula, D.K., Patel, S.K., Pathak, M., Patten, S.B., Patton, G.C., Pazoki Toroudi, H., Peden, A.E., Pennini, A., Pepito, V.C.F., Peprah, E.K., Pereira, D.M., Pesudovs, K., Pham, H.Q., Phillips, M.R., Piccinelli, C., Pilz, T.M., Piradov, M.A., Pirsaheb, M., Plass, D., Polinder, S., Polkinghorne, K.R., Pond, C.D., Postma, M.J., Pourjafar, H., Pourmalek, F., Poznańska, A., Prada, S.I., Prakash, V., Pribadi, D.R.A., Pupillo, E., Quazi Syed, Z., Rabiee, M., Rabiee, N., Radfar, A., Rafiee, A., Raggi, A., Rahman, M.A., Rajabpour-Sanati, A., Rajati, F., Rakovac, I., Ram, P., Ramezanzadeh, K., Ranabhat, C.L., Rao, P.C., Rao, S.J., Rashedi, V., Rathi, P., Rawaf, D.L., Rawaf, S., Rawal, L., Rawassizadeh, R., Rawat, R., Razo, C., Redford, S.B., Reiner, R.C., Reitsma, M.B., Remuzzi, G., Renjith, V., Renzaho, A.M.N., Resnikoff, S., Rezaei, Negar, Rezaei, Nima, Rezapour, A., Rhinehart, P.A., Riahi, S.M., Ribeiro, D.C., Ribeiro, D., Rickard, J., Rivera, J.A., Roberts, N.L.S., Rodríguez-Ramírez, S., Roever, L., Ronfani, L., Room, R., Roshandel, G., Roth, G.A., Rothenbacher, D., Rubagotti, E., Rwegerera, G.M., Sabour, S., Sachdev, P.S., Saddik, B., Sadeghi, E., Sadeghi, M., Saeedi, R., Saeedi Moghaddam, S., Safari, Y., Safi, S., Safiri, S., Sagar, R., Sahebkar, A., Sajadi, S.M., Salam, N., Salamati, P., Salem, H., Salem, M.R.R., Salimzadeh, H., Salman, O.M., Salomon, J.A., Samad, Z., Samadi Kafil, H., Sambala, E.Z., Samy, A.M., Sanabria, J., Sánchez-Pimienta, T.G., Santomauro, D.F., Santos, I.S., Santos, J.V., Santric-Milicevic, M.M., Saraswathy, S.Y.I., Sarmiento-Suárez, R., Sarrafzadegan, N., Sartorius, B., Sarveazad, A., Sathian, B., Sathish, T., Sattin, D., Saxena, S., Schaeffer, L.E., Schiavolin, S., Schlaich, M.P., Schmidt, M.I., Schutte, A.E., Schwebel, D.C., Schwendicke, F., Senbeta, A.M., Senthilkumaran, S., Sepanlou, S.G., Serdar, B., Serre, M.L., Shadid, J., Shafaat, O., Shahabi, S., Shaheen, A.A., Shaikh, M.A., Shalash, A.S., Shams-Beyranvand, M., Shamsizadeh, M., Sharafi, K., Sheikh, A., Sheikhtaheri, A., Shibuya, K., Shield, K.D., Shigematsu, M., Shin, J. Il, Shin, M.J., Shiri, R., Shirkoohi, R., Shuval, K., Siabani, S., Sierpinski, R., Sigfusdottir, I.D., Sigurvinsdottir, R., Silva, J.P., Simpson, K.E., Singh, J.A., Singh, P., Skiadaresi, E., Skou, S.T.S., Skryabin, V.Y., Smith, E.U.R., Soheili, A., Soltani, S., Soofi, M., Sorensen, R.J.D., Soriano, J.B., Sorrie, M.B., Soshnikov, S., Soyiri, I.N., Spencer, C.N., Spotin, A., Sreeramareddy, C.T., Srinivasan, V., Stanaway, J.D., Stein, C., Stein, D.J., Steiner, C., Stockfelt, L., Stokes, M.A., Straif, K., Stubbs, J.L., Sufiyan, M.B., Suleria, H.A.R., Suliankatchi Abdulkader, R., Sulo, G., Sultan, I., Szumowski, Ł., Tabarés-Seisdedos, R., Tabb, K.M., Tabuchi, T., Taherkhani, A., Tajdini, M., Takahashi, K., Takala, J.S., Tamiru, A.T., Taveira, N., Tehrani-Banihashemi, A., Temsah, M.H., Tesema, G.A., Tessema, Z.T., Thurston, G.D., Titova, M.V., Tohidinik, H.R., Tonelli, M., Topor-Madry, R., Topouzis, F., Torre, A.E., Touvier, M., Tovani-Palone, M.R.R., Tran, B.X., Travillian, R., Tsatsakis, A., Tudor Car, L., Tyrovolas, S., Uddin, R., Umeokonkwo, C.D., Unnikrishnan, B., Upadhyay, E., Vacante, M., Valdez, P.R., van Donkelaar, A., Vasankari, T.J., Vasseghian, Y., Veisani, Y., Venketasubramanian, N., Violante, F.S., Vlassov, V., Vollset, S.E., Vos, T., Vukovic, R., Waheed,

Y., Wallin, M.T., Wang, Y., Wang, Y.P., Watson, A., Wei, J., Wei, M.Y.W., Weintraub, R.G., Weiss, J., Werdecker, A., West, J.J., Westerman, R., Whisnant, J.L., Whiteford, H.A., Wiens, K.E., Wolfe, C.D.A., Wozniak, S.S., Wu, A.M., Wu, J., Wulf Hanson, S., Xu, G., Xu, R., Yadgir, S., Yahyazadeh Jabbari, S.H., Yamagishi, K., Yaminfirooz, M., Yano, Y., Yaya, S., Yazdi-Feyzabadi, V., Yeheyis, T.Y., Yilgwan, C.S., Yilma, M.T., Yip, P., Yonemoto, N., Younis, M.Z., Younker, T.P., Yousefi, B., Yousefi, Z., Yousefinezhadi, T., Yousuf, A.Y., Yu, C., Yusefzadeh, H., Zahirian Moghadam, T., Zamani, M., Zamanian, M., Zandian, H., Zastrozhin, M.S., Zhang, Y., Zhang, Z.J., Zhao, J.T., Zhao, X.J.G., Zhao, Y., Zhou, M., Ziapour, A., Zimsen, S.R.M., Brauer, M., Afshin, A., Lim, S.S., 2020. Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet 396, 1223–1249. https://doi.org/10.1016/S0140-6736(20)30752-2

Muschket, M., Di Paolo, C., Tindall, A.J., Touak, G., Phan, A., Krauss, M., Kirchner, K., Seiler, T.B., Hollert, H., Brack, W., 2018. Identification of Unknown Antiandrogenic Compounds in Surface Waters by Effect-Directed Analysis (EDA) Using a Parallel Fractionation Approach. Environ Sci Technol 52, 288–297. https://doi.org/10.1021/acs.est.7b04994

Neale, P.A., Ait-Aissa, S., Brack, W., Creusot, N., Denison, M.S., Deutschmann, B., Hilscherová, K., Hollert, H., Krauss, M., Novák, J., Schulze, T., Seiler, T.B., Serra, H., Shao, Y., Escher, B.I., 2015. Linking in Vitro Effects and Detected Organic Micropollutants in Surface Water Using Mixture-Toxicity Modeling. Environ Sci Technol 49, 14614–14624. https://doi.org/10.1021/acs.est.5b04083

Newton, S.R., Sobus, J.R., Ulrich, E.M., Singh, R.R., Chao, A., McCord, J., Laughlin-Toth, S., Strynar, M., 2020. Examining NTA performance and potential using fortified and reference house dust as part of EPA's Non-Targeted Analysis Collaborative Trial (ENTACT). Anal Bioanal Chem. https://doi.org/10.1007/s00216-020-02658-w

Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., Erwin, K.N., Levin, E.D., 2009. The toxicology of climate change: Environmental contaminants in a warming world. Environ Int 35, 971–986. https://doi.org/10.1016/j.envint.2009.02.006

Oberleitner, D., Schmid, R., Schulz, W., Bergmann, A., Achten, C., 2021. Feature-based molecular networking for identification of organic micropollutants including metabolites by non-target analysis applied to riverbank filtration.

Ottenbros, I., Lebret, E., Huber, C., Lommen, A., Antignac, J.P., Čupr, P., Šulc, L., Mikeš, O., Szigeti, T., Középesy, S., Martinsone, I., Martinsone, Z., Akulova, L., Pardo, O., Fernández, S.F., Coscollá, C., Pedraza-Diaz, S., Krauss, M., Debrauwer, L., Wagner, K., Nijssen, R., Mol, H., Vitale, C.M., Klanova, J., Molina, B.G., León, N., Vermeulen, R., Luijten, M., Vlaanderen, J., 2023. Assessment of exposure to pesticide mixtures in five European countries by a harmonized urinary suspect screening approach. Int J Hyg Environ Health 248. https://doi.org/10.1016/j.ijheh.2022.114105

Palm, E., Kruve, A., 2022. Machine Learning for Absolute Quantification of Unidentified Compounds in Non-Targeted LC/HRMS. Molecules 27. https://doi.org/10.3390/molecules27031013

Palmen, N.G.M., 2016. Early Warning Systems to Detect New and Emerging Risks in Europe. National Institute for Public Health and the Environment (RIVM). RIVM Letter 2016-0022.

Papazian, S., D'Agostino, L.A., Sadiktsis, I., Froment, J., Bonnefille, B., Sdougkou, K., Xie, H., Athanassiadis, I., Budhavant, K., Dasari, S., Andersson, A., Gustafsson, Ö., Martin, J.W., 2022.

Nontarget mass spectrometry and in silico molecular characterization of air pollution from the Indian subcontinent. Commun Earth Environ 3, 1–14. https://doi.org/10.1038/s43247-022-00365-1

Peets, P., Wang, W.C., Macleod, M., Breitholtz, M., Martin, J.W., Kruve, A., 2022. MS2Tox Machine Learning Tool for Predicting the Ecotoxicity of Unidentified Chemicals in Water by Nontarget LC-HRMS. Environ Sci Technol 56, 15508–15517. https://doi.org/10.1021/acs.est.2c02536

Perez-Riverol, Y., Moreno, P., 2020. Scalable Data Analysis in Proteomics and Metabolomics Using BioContainers and Workflows Engines. Proteomics 20, 1900147. https://doi.org/10.1002/pmic.201900147

Peter, K.T., Phillips, A.L., Knolhoff, A.M., Gardinali, P.R., Manzano, C.A., Miller, K.E., Pristner, M., Sabourin, L., Sumarah, M.W., Warth, B., Sobus, J.R., 2021. Nontargeted Analysis Study Reporting Tool: A Framework to Improve Research Transparency and Reproducibility. Anal Chem 93, 13870–13879. https://doi.org/10.1021/acs.analchem.1c02621

Petras, D., Minich, J.J., Cancelada, L.B., Torres, R.R., Kunselman, E., Wang, M., White, M.E., Allen, E.E., Prather, K.A., Aluwihare, L.I., Dorrestein, P.C., 2021. Non-targeted tandem mass spectrometry enables the visualization of organic matter chemotype shifts in coastal seawater. Chemosphere 129450. https://doi.org/10.1016/j.chemosphere.2020.129450

Pieke, E.N., Granby, K., Trier, X., Smedsgaard, J., 2017. A framework to estimate concentrations of potentially unknown substances by semi-quantification in liquid chromatography electrospray ionization mass spectrometry. Anal Chim Acta 975, 30–41. https://doi.org/10.1016/j.aca.2017.03.054

Plassmann, M.M., Fischer, S., Benskin, J.P., 2018. Nontarget Time Trend Screening in Human Blood. Environ Sci Technol Lett 5, 335–340. https://doi.org/10.1021/acs.estlett.8b00196

Pourchet, M., Debrauwer, L., Klanova, J., Price, E.J., Covaci, A., Caballero-Casero, N., Oberacher, H., Lamoree, M., Damont, A., Fenaille, F., Vlaanderen, J., Meijer, J., Krauss, M., Sarigiannis, D., Barouki, R., Le Bizec, B., Antignac, J.-P., 2020. Suspect and non-targeted screening of chemicals of emerging concern for human biomonitoring, environmental health studies and support to risk assessment: From promises to challenges and harmonisation issues. Environ Int 139, 105545. https://doi.org/10.1016/j.envint.2020.105545

Pozo, O.J., Deventer, K., Van Eenoo, P., Delbeke, F.T., 2008. Efficient Approach for the Comprehensive Detection of Unknown Anabolic Steroids and Metabolites in Human Urine by Liquid Chromatography−Electrospray-Tandem Mass Spectrometry. Anal Chem 80, 1709–1720. https://doi.org/10.1021/ac7020757

Price, P.S., Jarabek, A.M., Burgoon, L.D., 2020. Organizing mechanism-related information on chemical interactions using a framework based on the aggregate exposure and adverse outcome pathways. Environ Int 138, 105673. https://doi.org/10.1016/j.envint.2020.105673

Pruvost-Couvreur, M., Le Bizec, B., Béchaux, C., Rivière, G., 2020a. Dietary risk assessment methodology: how to deal with changes through life. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 37, 705–722. https://doi.org/10.1080/19440049.2020.1727964

Pruvost-Couvreur, M., Le Bizec, B., Margaritis, I., Volatier, J.L., Béchaux, C., Rivière, G., 2020b. Impact of dietary guidelines on lifetime exposure to chemical contaminants: Divergent conclusions for

two bioaccumulative substances. Food and Chemical Toxicology 145. https://doi.org/10.1016/j.fct.2020.111672

Quiros-alcala, L., Barr, D.B., 2023. Invited Perspective Invited Perspective : Mixtures — Are They Worth the Risk ( Assessment )? 131, 1–2.

Rampler, E., Abiead, Y. El, Schoeny, H., Rusz, M., Hildebrand, F., Fitz, V., Koellensperger, G., 2021. Recurrent Topics in Mass Spectrometry-Based Metabolomics and Lipidomics - Standardization, Coverage, and Throughput. Anal Chem 93, 519–545. https://doi.org/10.1021/acs.analchem.0c04698

Ribas Batlle, M., Bernard, T., Brill, E., Coelho, M.R., Coimbra, M.F., Deuerlein, J., Gattinesi, P., Hohenblum, P., Pieronne, P., Raich, J., Simas, L., Teixeira, R., Ugarelli, R., Weingartner, A., Cardarilli, M., Giannopoulos, G., 2022. Water security plan: Towards a more resilient drinking water infrastructure.

Rivier, L., 2003. Criteria for the identification of compounds by liquid chromatography-mass spectrometry and liquid chromatography-multiple mass spectrometry in forensic toxicology and doping analysis. Anal Chim Acta 492, 69–82. https://doi.org/10.1016/S0003-2670(03)00889-4

Rochat, B., 2017. Proposed Confidence Scale and ID Score in the Identification of Known-Unknown Compounds Using High Resolution MS Data. J Am Soc Mass Spectrom 28, 709–723. https://doi.org/10.1007/s13361-016-1556-0

Ruff, M., Mueller, M.S., Loos, M., Singer, H.P., 2015. Quantitative target and systematic non-target analysis of polar organic micro-pollutants along the river Rhine using high-resolution mass-spectrometry - Identification of unknown sources and compounds. Water Res 87, 145–154. https://doi.org/10.1016/j.watres.2015.09.017

Salek, R.M., Steinbeck, C., Viant, M.R., Goodacre, R., Dunn, W.B., 2013. The role of reporting standards for metabolite annotation and identification in metabolomic studies. Gigascience 2, 1. https://doi.org/10.1186/2047-217X-2-13

Sandra, E.R., Christophe, A., Tabet, J.J., 2016. High resolution mass spectrometry for structural identification of metabolites in metabolomics. Metabolomics 12, 1–15. https://doi.org/10.1007/s11306-015-0882-8

Savitz, D.A., Hattersley, A.M., 2023. Evaluating Chemical Mixtures in Epidemiological Studies to Inform Regulatory Decisions. Environ Health Perspect 131, 45001. https://doi.org/10.1289/EHP11899

Sawada, Y., Akiyama, K., Sakata, A., Kuwahara, A., Otsuki, H., Sakurai, T., Saito, K., Hirai, M.Y., 2009. Widely targeted metabolomics based on large-scale MS/MS data for elucidating metabolite accumulation patterns in plants. Plant Cell Physiol 50, 37–47. https://doi.org/10.1093/pcp/pcn183

Schrimpe-Rutledge, A.C., Codreanu, S.G., Sherrod, S.D., McLean, J.A., 2016. Untargeted Metabolomics Strategies—Challenges and Emerging Directions. J Am Soc Mass Spectrom 27, 1897–1905. https://doi.org/10.1007/s13361-016-1469-y

Schulze, T., Schymanski, E., Stravs, M., Neumann, S., Krauss, M., Singer, H., Gallampois, C., Hug, C., Hollender, Juliane, Slobodnik, J., Brack, W., 2012. NORMAN MassBank Towards a community-

driven, open-access accurate mass spectral database for the identifi cation of emerging pollutants. NORMAN Network Bulletin 1–20.

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014a. Identifying small molecules via high resolution mass spectrometry: Communicating confidence. Environ Sci Technol 48, 2097–2098. https://doi.org/10.1021/es5002105

Schymanski, E.L., Singer, H.P., Longrée, P., Loos, M., Ruff, M., Stravs, M.A., Ripollés Vidal, C., Hollender, J., 2014b. Strategies to characterize polar organic contamination in wastewater: Exploring the capability of high resolution mass spectrometry. Environ Sci Technol 48, 1811–1818. https://doi.org/10.1021/es4044374

Shafi, A., Berry, A.J., Sumnall, H., Wood, D.M., Tracy, D.K., 2020. New psychoactive substances: a review and updates. Ther Adv Psychopharmacol 10, 204512532096719. https://doi.org/10.1177/2045125320967197

Simon, E., Lamoree, M.H., Hamers, T., de Boer, J., 2015. Challenges in effect-directed analysis with a focus on biological samples. TrAC - Trends in Analytical Chemistry 67, 179–191. https://doi.org/10.1016/j.trac.2015.01.006

Sims, K., 2022. Chemicals of concern: a prioritisation and early warning system for England. Royal Society of Chemistry—Environmental Chemistry Group—Bulletin ECG Environmental Brief No.35.

Sobus, J.R., Wambaugh, J.F., Isaacs, K.K., Williams, A.J., McEachran, A.D., Richard, A.M., Grulke, C.M., Ulrich, E.M., Rager, J.E., Strynar, M.J., Newton, S.R., 2018. Integrating tools for non-targeted analysis research and chemical safety evaluations at the US EPA. J Expo Sci Environ Epidemiol 28, 411–426. https://doi.org/10.1038/s41370-017-0012-y

Spicer, R.A., Salek, R., Steinbeck, C., 2017. Compliance with minimum information guidelines in public metabolomics repositories. Sci Data 4, 1–8. https://doi.org/10.1038/sdata.2017.137

Steiner, D., Sulyok, M., Malachová, A., Mueller, A., Krska, R., 2020. Realizing the simultaneous liquid chromatography-tandem mass spectrometry based quantification of >1200 biotoxins, pesticides and veterinary drugs in complex feed. J Chromatogr A 1629, 461502. https://doi.org/10.1016/j.chroma.2020.461502

Sud, M., Fahy, E., Cotter, D., Azam, K., Vadivelu, I., Burant, C., Edison, A., Fiehn, O., Higashi, R., Nair, K.S., Sumner, S., Subramaniam, S., 2016. Metabolomics Workbench: An international repository for metabolomics data and metadata, metabolite standards, protocols, tutorials and training, and analysis tools. Nucleic Acids Res 44, D463–D470. https://doi.org/10.1093/nar/gkv1042

Sumner, L.W., Amberg, A., Barrett, D., Beale, M.H., Beger, R., Daykin, C. a., Fan, T.W.-M., Fiehn, O., Goodacre, R., Griffin, J.L., Hankemeier, T., Hardy, N., Harnly, J., Higashi, R., Kopka, J., Lane, A.N., Lindon, J.C., Marriott, P., Nicholls, A.W., Reily, M.D., Thaden, J.J., Viant, M.R., 2007. Proposed minimum reporting standards for chemical analysis. Metabolomics 3, 211–221. https://doi.org/10.1007/s11306-007-0082-2

Sumner, L.W., Lei, Z., Nikolau, B.J., Saito, K., Roessner, U., Trengove, R., 2014. Proposed quantitative and alphanumeric metabolite identification metrics. Metabolomics 10, 1047–1049. https://doi.org/10.1007/s11306-014-0739-6

Theodoridis, G., Gika, H., Raftery, D., Goodacre, R., Plumb, R.S., Wilson, I.D., 2023. Ensuring Fact-Based Metabolite Identification in Liquid Chromatography-Mass Spectrometry-Based Metabolomics. Anal Chem 95, 3909–3916. https://doi.org/10.1021/acs.analchem.2c05192

Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., Gilbreath, A., Sutton, R., Scholz, N.L., Davis, J.W., Dodd, M.C., Simpson, A., McIntyre, J.K., Kolodziej, E.P., 2021. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science (1979) 371, 185–189. https://doi.org/10.1126/science.abd6951

Trudsø, L.L., Nielsen, M.B., Hansen, S.F., Syberg, K., Kampmann, K., Khan, F.R., Palmqvist, A., 2022. The need for environmental regulation of tires: Challenges and recommendations. Environmental Pollution 311. https://doi.org/10.1016/j.envpol.2022.119974

Ulrich, E.M., Sobus, J.R., Grulke, C.M., Richard, A.M., Newton, S.R., Strynar, M.J., Mansouri, K., Williams, A.J., 2019. EPA's non-targeted analysis collaborative trial (ENTACT): genesis, design, and initial findings. Anal Bioanal Chem 411, 853–866. https://doi.org/10.1007/s00216-018-1435-6

Vandenberg, L.N., Rayasam, S.D.G., Axelrad, D.A., Bennett, D.H., Brown, P., Carignan, C.C., Chartres, N., Diamond, M.L., Joglekar, R., Shamasunder, B., Shrader-Frechette, K., Subra, W.A., Zarker, K., Woodruff, T.J., 2023. Addressing systemic problems with exposure assessments to protect the public's health. Environ Health. https://doi.org/10.1186/s12940-022-00917-0

Vermeulen, R., Schymanski, E.L., Barabási, A.-L., Miller, G.W., 2020. The exposome and health: Where chemistry meets biology. Science 367, 392–396. https://doi.org/10.1126/science.aay3164

Viegas, S., Jeddi, M.Z., Hopf, N.B., Bessems, J., Palmen, N., Galea, K.S., Jones, K., Kujath, P., Duca, R.C., Verhagen, H., Santonen, T., Pasanen-Kase, R., 2020. Biomonitoring as an underused exposure assessment tool in occupational safety and health context—challenges and way forward. Int J Environ Res Public Health 17, 1–20. https://doi.org/10.3390/ijerph17165884

Vitale, C.M., Lommen, A., Huber, C., Wagner, K., Garlito Molina, B., Nijssen, R., Price, E.J., Blokland, M., van Tricht, F., Mol, H.G.J., Krauss, M., Debrauwer, L., Pardo, O., Leon, N., Klanova, J., Antignac, J., 2022. Harmonized Quality Assurance/Quality Control Provisions for Nontargeted Measurement of Urinary Pesticide Biomarkers in the HBM4EU Multisite SPECIMEn Study. Anal Chem 94, 7833–7843. https://doi.org/10.1021/acs.analchem.2c00061

Vitale, C.M., Price, E.J., Miller, G.W., David, A., Antignac, J.-P., Barouki, R., Klánová, J., 2021. Analytical strategies for chemical exposomics: exploring limits and feasibility. Exposome 1–47. https://doi.org/10.1093/exposome/osab003

Vorkamp, K., Esteban López, M., Gilles, L., Göen, T., Govarts, E., Hajeb, P., Katsonouri, A., Knudsen, L.E., Kolossa-Gehring, M., Lindh, C., Nübler, S., Pedraza-Díaz, S., Santonen, T., Castaño, A., 2023. Coordination of chemical analyses under the European Human Biomonitoring Initiative (HBM4EU): Concepts, procedures and lessons learnt. Int J Hyg Environ Health 251. https://doi.org/10.1016/j.ijheh.2023.114183

Wang, M., Carver, J.J., Phelan, V. V., Sanchez, L.M., Garg, N., Peng, Y., Nguyen, D.D., Watrous, J., Kapono, C.A., Luzzatto-Knaan, T., Porto, C., Bouslimani, A., Melnik, A. V., Meehan, M.J., Liu, W.T., Crüsemann, M., Boudreau, P.D., Esquenazi, E., Sandoval-Calderón, M., Kersten, R.D., Pace, L.A., Quinn, R.A., Duncan, K.R., Hsu, C.C., Floros, D.J., Gavilan, R.G., Kleigrewe, K., Northen, T., Dutton,

R.J., Parrot, D., Carlson, E.E., Aigle, B., Michelsen, C.F., Jelsbak, L., Sohlenkamp, C., Pevzner, P., Edlund, A., McLean, J., Piel, J., Murphy, B.T., Gerwick, L., Liaw, C.C., Yang, Y.L., Humpf, H.U., Maansson, M., Keyzers, R.A., Sims, A.C., Johnson, A.R., Sidebottom, A.M., Sedio, B.E., Klitgaard, A., Larson, C.B., Boya, C.A.P., Torres-Mendoza, D., Gonzalez, D.J., Silva, D.B., Marques, L.M., Demarque, D.P., Pociute, E., O'Neill, E.C., Briand, E., Helfrich, E.J.N., Granatosky, E.A., Glukhov, E., Ryffel, F., Houson, H., Mohimani, H., Kharbush, J.J., Zeng, Y., Vorholt, J.A., Kurita, K.L., Charusanti, P., McPhail, K.L., Nielsen, K.F., Vuong, L., Elfeki, M., Traxler, M.F., Engene, N., Koyama, N., Vining, O.B., Baric, R., Silva, R.R., Mascuch, S.J., Tomasi, S., Jenkins, S., Macherla, V., Hoffman, T., Agarwal, V., Williams, P.G., Dai, J., Neupane, R., Gurr, J., Rodríguez, A.M.C., Lamsa, A., Zhang, C., Dorrestein, K., Duggan, B.M., Almaliti, J., Allard, P.M., Phapale, P., Nothias, L.F., Alexandrov, T., Litaudon, M., Wolfender, J.L., Kyle, J.E., Metz, T.O., Peryea, T., Nguyen, D.T., VanLeer, D., Shinn, P., Jadhav, A., Müller, R., Waters, K.M., Shi, W., Liu, X., Zhang, L., Knight, R., Jensen, P.R., Palsson, B., Pogliano, K., Linington, R.G., Gutiérrez, M., Lopes, N.P., Gerwick, W.H., Moore, B.S., Dorrestein, P.C., Bandeira, N., 2016. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. Nat Biotechnol 34, 828–837. https://doi.org/10.1038/nbt.3597

Wang, Z., Walker, G.W., Muir, D.C.G., Nagatani-Yoshida, K., 2020a. Toward a Global Understanding of Chemical Pollution: A First Comprehensive Analysis of National and Regional Chemical Inventories. Environ Sci Technol 54, 2575–2584. https://doi.org/10.1021/acs.est.9b06379

Wang, Z., Walker, G.W., Muir, D.C.G., Nagatani-Yoshida, K., 2020b. Toward a Global Understanding of Chemical Pollution: A First Comprehensive Analysis of National and Regional Chemical Inventories. Environ Sci Technol 54, 2575–2584. https://doi.org/10.1021/acs.est.9b06379

Wicker, J., Lorsbach, T., Gütlein, M., Schmid, E., Latino, D., Kramer, S., Fenner, K., 2016. enviPath – The environmental contaminant biotransformation pathway resource. Nucleic Acids Res 44, D502–D508. https://doi.org/10.1093/nar/gkv1229

World Health Organization, 2022a. Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda.

World Health Organization, 2022b. Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda.

Yuan, H., Cao, G., Hou, X., Huang, M., Du, P., Tan, T., Zhang, Y., Zhou, H., Liu, X., Liu, L., Jiangfang, Y., Li, Y., Liu, Z., Fang, C., Zhao, L., Fernie, A.R., Luo, J., 2022. Development of a widely targeted volatilomics method for profiling volatilomes in plants. Mol Plant 15, 189–202. https://doi.org/10.1016/j.molp.2021.09.003

Zare Jeddi, M., Hopf, N.B., Louro, H., Viegas, S., Galea, K.S., Pasanen-Kase, R., Santonen, T., Mustieles, V., Fernandez, M.F., Verhagen, H., Bopp, S.K., Antignac, J.P., David, A., Mol, H., Barouki, R., Audouze, K., Duca, R.C., Fantke, P., Scheepers, P., Ghosh, M., Van Nieuwenhuyse, A., Lobo Vicente, J., Trier, X., Rambaud, L., Fillol, C., Denys, S., Conrad, A., Kolossa-Gehring, M., Paini, A., Arnot, J., Schulze, F., Jones, K., Sepai, O., Ali, I., Brennan, L., Benfenati, E., Cubadda, F., Mantovani, A., Bartonova, A., Connolly, A., Slobodnik, J., Bruinen de Bruin, Y., van Klaveren, J., Palmen, N., Dirven, H., Husøy, T., Thomsen, C., Virgolino, A., Röösli, M., Gant, T., von Goetz, N., Bessems, J., 2022. Developing human biomonitoring as a 21st century toolbox within the European exposure science strategy 2020–2030. Environ Int 168. https://doi.org/10.1016/j.envint.2022.107476

Zhang, X., Di Lorenzo, R.A., Helm, P.A., Reiner, E.J., Howard, P.H., Muir, D.C.G., Sled, J.G., Jobst, K.J., 2019. Compositional space: A guide for environmental chemists on the identification of persistent and bioaccumulative organics using mass spectrometry. Environ Int 132, 104808. https://doi.org/10.1016/j.envint.2019.05.002

Zhao, F., Li, L., Chen, Y., Huang, Y., Keerthisinghe, T.P., Chow, A., Dong, T., Jia, S., Xing, S., Warth, B., Huan, T., Fang, M., 2021. Risk-based chemical ranking and generating a prioritized human exposome database. Environ Health Perspect 129, 1–12. https://doi.org/10.1289/EHP7722

Zhou, J., Yin, Y., 2016. Strategies for large-scale targeted metabolomics quantification by liquid chromatography-mass spectrometry. Analyst 141, 6362–6373. https://doi.org/10.1039/c6an01753c

Zomer, P., Mol, H.G.J., 2015. Simultaneous quantitative determination, identification and qualitative screening of pesticides in fruits and vegetables using LC-Q-Orbitrap™-MS. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 32, 1628–1636. https://doi.org/10.1080/19440049.2015.1085652

Zwart, N., Nio, S.L., Houtman, C.J., De Boer, J., Kool, J., Hamers, T., Lamoree, M.H., 2018. High-Throughput Effect-Directed Analysis Using Downscaled in Vitro Reporter Gene Assays to Identify Endocrine Disruptors in Surface Water. Environ Sci Technol 52, 4367–4377. https://doi.org/10.1021/acs.est.7b06604mailto:ziga.tkalec@recetox.muni.czmailto:jurij.trontej@ffa.uni-lj.si