Full length article

Innovative analytical methodologies for characterizing chemical exposure with a view to next-generation risk assessment

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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.

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 characterization 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 analyzed 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 recognized (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 harmonize conventional internal exposure characterization, 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 characterization 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 analytical methods for enhanced characterization 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 scientific barriers that need to be overcome in this process and provide recommendations for building a sustainable European capacity to progress the use of innovative analytical methodologies to improve the characterization of chemical exposure in a regulatory context.

1.1. 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 underutilized 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 prioritized 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 analyzing 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 favor 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 operates 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 optimize transitions, with greater selectivity favoring 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 prior defined 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 are user prescribed. Detected features will share a common defined substructure (e.g., glucuronide, hydroxyphenyl etc.), but the number of analytes detected will be unknown and not fully dependent on user input. Precursor-ion scanning methods have been widely applied e.g., to detect unknown anabolic steroids during anti-doping testing (Pozo et al., 2008)..

Lastly, we deem suspect screening approaches to be a subset of non-targeted procedures, whereby the resultant data is then selectively analyzed using user-defined ions that are specific for known chemicals

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.

(Krauss et al., 2010), or diagnostic of substructures of interest i.e., pre-determined, 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).

A key benefit of non-targeted acquisition is that the generated data can be archived and re-analyzed as more information and resources become available in future. For example, within the HBM4EU Survey on Pesticide Mixtures in Europe (SPECIMEn) study, a suspect screen matched the molecular formulas – generated from isotopic pattern analysis of features detected in urine - with a curated database of >4600 pesticides and their potential metabolites, 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 and performed the retrospective absolute quantification of 395 detected analytes via standard addition calibration (Gago-Ferrero et al., 2020). As another example, 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) was used 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.

1.1.1. 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 bio-accumulation 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. Therefore, although substances newly introduced in commerce will be deemed safer, they will also be more challenging to distinguish. As a result, the mechanisms underlying unforeseen adverse effects may become harder to elucidate.

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 prioritized (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.

1.1.2. 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. Recently, a database integrating >2 million natural and synthetic chemicals into a global network, representing hypothetical biochemical space, has been constructed (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.

1.2. Barrier 2: Analyte quantification

Although the main bottleneck of exploratory analyzes 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 the confirmatory identification, estimation of concentration in samples, and associated measurement uncertainty, 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 analyzes remain required for exposure risk assessment and to support risk management decision making (Mccord et al., 2022).

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) (Groff et al., 2022). Typically, the concentrations of 90 % of detected features can be predicted within two orders of magnitude of the true values. 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, ionization efficiency (IE)-based procedures for electrospray ionization 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 (Palm and Kruve, 2022) or fragmentation patterns (Sepman et al., 2023) without structural assignments, extending the approach to quantify unidentified features. That said, IE prediction models require training on large collections of empirical ionization response data for analytes representative of the

intended chemical space. Limited IE data is currently available, hindering the accuracy and applicability.

Although the concentration of unknown features is disconnected from hazard risk, it can support feature prioritization 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 with significant uncertainty that should be communicated for each prediction of each chemical (Fisher et al., 2022). In particular, the use of an upper confidence limit of quantitative estimates, i.e., reflecting uncertainty, has been demonstrated and can serve as a protective concentration estimate suitable for provisional risk assessment (Groff et al., 2022). However, the validity of the confidence limits depends on the surrogate analyte data being representative, an assumption that will not always be met (Pu et al., 2024), and further comparative studies needed to assess the validity of different estimation approaches.

Although estimating analyte concentrations in absence of reference standards is not yet widely implemented, it shows promise to bridge the gap between conventional monitoring and exploratory approaches for chemical exposure characterization.

1.3. Barrier 3: Analyte prioritization

The tentative annotation of hundreds to thousands of features means prioritization 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 temporal and spatial 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. The 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. Since 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. In another example, predicted dose-to-concentration ratios were used to prioritize features of highest bioaccumulation potential based solely on proposed molecular formulae (Zhang et al., 2023).

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 the accuracy and transferability.

1.3.1. Bioactivity-based prioritization

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 prioritization 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 prioritization 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.

When specific effects are of interest, fractionation can be replaced with focused extractions e.g., affinity purification via pull-down assays, constraining detection to features representing analytes that interact with target receptor proteins. Though lower throughput, these approaches show promise as the reduced complexity of extracts undergoing MS analysis aids subsequent feature identification.

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 prioritized 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, and 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 mitigate these restrictions at least partly. 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 prioritization 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* assays is promising (Brion et al., 2019).

1.4. Barrier 4. Reporting

The diversity of approaches applied for characterization 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 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).

1.4.1. Identification confidence

Various criteria are available for monitoring of *a priori* known substances to ensure accurate reporting, via confirming observed physico-chemical 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.

1.4.2. 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 (Fisher et al., 2022). 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 formalized 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-characterized 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 all 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 are dependent on a high degree of initial sample characterization 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 standardized 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/), favored to leverage, extend and cooperate with an established developer and user community for small molecule mass spectrometry data processing.

Establishing and adhering to harmonized 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.

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

The identification and prioritization of potentially hazardous chemicals are critical components of an Early Warning System (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.

1.5.1. 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 Organization (WHO) Water Safety Plans has been recommended (World Health Organization, 2022).

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) has outlined a structure for the incorporation of non-targeted analysis to support identification and risk-based chemical prioritization 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 characterzing 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).

1.5.2. 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, with importance for the identification of potential new or emerging work-related diseases recognized (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 decentralized network of laboratories hindered data comparability and aggregation, particularly when laboratories reported considerably different detection frequencies (Vorkamp et al., 2023). The challenges of decentralized 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 harmonized, multi-site suspect screening procedures being evidenced, e.g., SPEC-IMEn (Ottenbros et al., 2023), the complexity of associated data processing remains a bottleneck.

2. Scientific recommendations to advance the use of innovative analytical methodologies for chemical risk assessment

2.1. 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, spectra and metadata 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.

2.2. 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/

Table 2

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

Identification status	Approach	Relative quantitative uncertainty	Relative 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
	Response factor bounding	High	Medium

[a] Ionization efficiency.

miscommunication. A repository for community provision of ionization efficiency data has been set up and collection of information about ionization efficiency should be implemented into reference mass spectral databases (Liigand et al., 2021).

2.3. 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 prioritization 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 parallelization of fractionation and miniaturization 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 as the implementation of *in vitro* to *in vivo* extrapolation (IVIVE) approach will be needed to increase the relevance of feature prioritization based on toxicity. Finally, because EDA is time consuming, a two-step strategy consisting firstly of mass balance calculation, allowing to unravel if know chemical explained the detected activity, followed by fractionation seems relevant.

2.4. 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.

Harmonized data processing and annotation workflows should be adopted to support comparability of generated results. This is especially vital for multi-center 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 are 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. Controlled vocabularies for uniform descriptive reporting should be developed to enable automated report aggregation. Adhering to these reporting requirements promotes transparency, reproducibility, and comparability of results across different studies and laboratories.

2.5. 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 EWS or regulatory entity should be established to ensure that only confirmed, or sufficiently identified substances, are escalated.

The feasibility of integrating 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 concerns.

A network of harmonized, qualified monitoring laboratories should be implemented to provide capacity for large-scale monitoring. Select analytical workflows should be standardized 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, plus the NORMAN network. Furthermore, building on cooperations within the Horizon 2020 initiative project 'Exposome powered tools for healthy living in urban settings' (EXPANSE) and the Horizon Europe initiative project 'Discovering the Causes of Three Poorly Understood Cancers in Europe' (DISCERN), the EIRENE RI has commenced a pilot EU-US harmonization 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.

3. 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 analytical 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 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.

Though we have focused on innovative analytical methods, the tandem development of innovative computational approaches for exposure characterization, hazard characterization and their integration with empirical data is paramount to support next-generation risk assessment (Egeghy et al., 2016). Innovative high-throughput exposure (HTE) models and toxicokinetic methods capitalize on computational advances for the estimation of chemical exposure at populational and individual levels (Wambaugh et al., 2019). Such approaches are essential due to the dearth of empirical information available for most chemicals, and to maximize the value of limited environmental and biomonitoring data. For example, the EPA's Systematic Empirical Evaluation of Models (SEEM) framework uses a consensus modelling method that combines predictions of chemical intake from HTE models with reconstructed exposure estimates based on biomonitoring data.

Evidencing the value of this approach, a SEEM meta-model based on biomonitoring measures of just 114 chemicals was used for the rapid estimation of median human intake rates for nearly 480 000 chemicals (Ring et al., 2019). In another example, the human blood concentrations of nearly 8000 chemicals were predicted via a random forest model trained and tested on 216 chemicals, and markedly, many of the toxicokinetic parameters input to the model were also estimated in silico (Zhao et al., 2023).

Moreover, the development of new approach methodologies (NAMs) for chemical hazard characterization has been placed onto numerous national strategic roadmaps and the obstacles, plus potential solutions, for integration in regulatory chemical risk assessment recently reviewed (Schmeisser et al., 2023). Notably, the National Toxicity Programme of the U.S. Department of Health and Human Services provides access to computational tools, including physiologically based pharmacokinetic (PBPK) and IVIVE models, alongside data generated via quantitative structure–activity relationship (QSAR) models based on the EPA's Open (quantitative) structure–activity Relationship App (OPERA) (Mansouri et al., 2018), and high-throughput screening within the Toxicology in the 21st Century program (Tox21) (Thomas et al., 2018), via its Integrated Chemical Environment (ICE) web platform (Abedini et al., 2021). ICE facilitates data and model transparency to enhance confidence in NAMs and encourage adoption in regulatory context. PARC activities dedicated to NAMs have been summarized and are being undertaken in close linkage with the development of AOPs, read-across and Integrated Approaches to Testing and Assessment (IATA) frameworks (De Castelbajac et al., 2023).

Similarly to ICE, the building of an overarching PARC computational tool and network infrastructure to support a harmonized framework for models and data is envisioned (Marx-Stoelting et al., 2023), aiming to drive a unified advance of computational and empirical chemical exposure and hazard characterization on the road to next-generation chemical risk assessment.

CRediT authorship contribution statement

Žiga Tkalec: Writing – review & editing, Writing – original draft. **Jean-Philippe Antignac:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Nicole Bandow:** Writing – review & editing. **Frederic M. Béen:** Writing – review & editing. **Lidia Belova:** Writing – review & editing. **Jos Bessems:** Writing – review & editing. **Bruno Le Bizec:** Writing – review & editing. **Werner Brack:** Writing – review & editing. **German Cano-Sancho:** Writing – review & editing. **Jade Chaker:** Writing – review & editing. **Adrian Covaci:** Writing – review & editing. **Nicolas Creusot:** Writing – review & editing. **Arthur David:** Writing – review & editing. **Laurent Debrauwer:** Writing – review & editing. **Gaud Dervilly:** Writing – review & editing. **Radu Corneliu Duca:** Writing – review & editing. **Valérie Fessard:** Writing - review & editing, Project administration, Funding acquisition. **Joan O. Grimalt:** Writing – review & editing. **Thierry Guerin:** Writing – review & editing. **Baninia Habchi:** Writing – review & editing. **Helge Hecht:** Writing – review & editing. **Juliane Hollender:** Writing – review & editing. **Emilien L. Jamin:** Writing – review & editing. **Jana Klánová:** Writing – review & editing. **Tina Kosjek:** Writing – review & editing. **Martin Krauss:** Writing – review & editing. **Marja Lamoree:** Project administration, Funding acquisition, Conceptualization. **Gwenaelle Lavison-Bompard:** Writing – review & editing. **Jeroen Meijer:** Writing – review & editing. **Ruth Moeller:** Writing – review & editing. **Hans Mol:** Writing – review & editing. **Sophie Mompelat:** Writing – review & editing. **An Van Nieuwenhuyse:** Writing – review & editing. **Herbert Oberacher:** Writing – review & editing. **Julien Parinet:** Writing – review & editing. **Christof Van Poucke:** Writing – review & editing. **Robert Roškar:** Writing – review & editing. **Anne Togola:** Writing – review & editing, Funding acquisition. **Jurij Trontelj:** Writing – review & editing. **Elliott J. Price:** Writing – review & editing, Writing – original draft, Supervision.

Declaration of competing interest

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.

Data availability

No data was used for the research described in the article.

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