Exposure Assessment of Emerging Chemicals and Novel Screening Strategies

Qingyang Zhu and Haixia Dai

Abstract Persistent organic pollutants (POPs) commonly exist in various kinds of environmental mediums and can migrant into plant food sources and bioaccumulate in the fatty tissues of human body. Traditional approach to evaluate POPs in multiple biofluids is based on targeted analytic chemistry. Recently, the development of sophisticated analytical instruments (e.g., tandem mass spectrometry, MS–MS) has provided the opportunity to quantify and identify chemical compounds to achieve good sensitivity and selectivity. In this chapter, we discuss the current assessment tools of chemical pollutants, including classic targeted approaches and novel untargeted methods. Targeted biomonitoring studies typically focused on a specific group of interest chemicals such as phthalate, bisphenol A (BPA), and polybrominated diphenyl ethers (PBDEs). Recent studies tended to use noninvasive or less-invasive bio-matrices which could be accessible in sufficient amounts for the analysis and do not pose a health risk for the donor. There does not exist an ideal matrix for universal situations, but depending on the toxicokinetic of the targeted chemical. Exposome includes a series of quantitative and repeated metrics of both endogenous and exogenous exposures that describe, holistically, environmental influences or exposure over a lifetime. At the current stage, the exposome is still in its infancy. Many technical and statistical challenges remain unsolved. Combined with data mining, via a series of statistical approaches, exposome shows great potential in identifying markers that can further lead to targeted analyses.

Keywords Persistent organic pollutants · Exposure assessment · Phthalates · Bisphenol A · Polybrominated diphenyl ethers · Exposome

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1 Introduction

Persistent organic pollutants (POPs) commonly exist in various kinds of environmental mediums. Due to their high resistance to degradation and high persistence in the environment, POPs can migrate into plant food sources from the environment and then bioaccumulate in the fatty tissues of human body with potential adverse health effect [1].

The traditional and the most widely used approach to evaluate POPs in multiple biofluids is based on targeted analytic chemistry. Recently, the development of sophisticated analytical instruments (e.g., tandem mass spectrometry, MS–MS) has provided the opportunity to quantify and identify chemical compounds to achieve good sensitivity and selectivity. However, targeted approach inevitably ignores the presence of other POPs contaminants since it requires standard references and analyze specific information. As a result, it is not able to give an exhaustive overview of other organic components in the biofluids. Full-spectrum acquisition techniques that provide accurate high resolution mass spectrometry, such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), show great potential for obtaining information on large amounts of organic compounds in human body.

In 2005, Wild proposed the conception of exposome, representing the totality of exposures throughout the lifespan [2]. Research efforts have soon begun after the definition was originally made, leading to a revised working definition that the exposome represents the cumulative measure of exposures to both chemical and nonchemical agents such as diet, stress, and socio-demographic factors. Exposome includes a series of quantitative and repeated metrics of both endogenous and exogenous exposures that describe, holistically, environmental influences or exposure over a lifetime. It means that not only the traditional measures of exposure (e.g., traditional biomonitoring, environmental monitoring) but also untargeted discovery of unknown chemicals of biological importance are included [3, 4]. Going a step beyond traditional biomonitoring, exposomic approaches aim to capture all exposures that potentially affect health and disease. Biomonitoring serves as the key tool to define exposure disease risks given the biological significance of internal exposure measurements. With the development of modern technology, novel screening strategies are critical in achieving a comprehensive understanding of exposures that are vital in personal and public health. Full understandings of the complex interactions between genetics and environmental exposures may greatly help to solve the mysteries of the epidemiology, trends, and prevention of many diseases.

We are only beginners to understand the complexities of environmental exposures and their impacts on human health. At present, we have limited estimates of the impact of environmental exposures on health, and uncertainty greatly exists in those [5]. In this chapter, we are going to discuss the current assessment tools of chemical pollutants, including classic targeted approaches and novel un-targeted methods. In addition, the current progress of exposome studies as well as its screening strategies are described here.

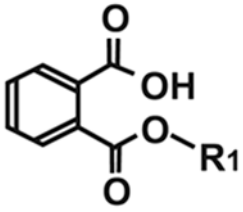
2 Targeted Approaches

Traditional biological measurements, also called targeted analyses, measure a target chemical, metabolite or reaction product in biofluids such as urine or blood. In epidemiological studies, targeted approaches have become a key component of exposure assessment to characterize biologically persistent chemicals, whereas short-lived chemicals are effectively measured only if the individual is undergoing continuous exposure, if the exposure timing is known. The utility of targeted methods in characterizing phthalates, bisphenol A, and PBDEs are discussed in this section.

1. **Phthalates**

Phthalates, the diesters of 1,2-benzendedicarboxylic acid (phthalic acid), are a class of synthetic chemicals that are used widely in industrial applications. Human exposure is common due to the widespread use of phthalates in commodities [6]. Ingestion, inhalation, and dermal contact are considered important routes of phthalates exposure for the general population, but the sources, pathways and routes may vary by phthalates due to the differences in their utility in products or their chemical and physical properties [7]. Phthalates have short biologic half-lives and are quickly excreted from the body.

Previous studies mostly focus on seven specific kinds of phthalates: (1) diethyl phthalate (DEP); (2) di-n-butyl phthalate (DBP); (3) di-isobutyl phthalate (DiBP); (4) butyl benzyl phthalate (BBzP); (5) di(2-ethylhexyl) phthalate (DEHP); (6) di-isononyl phthalate (DiNP); and (7) di-isodecyl phthalate (DiDP). Their chemical structures are shown in Fig. 1. There are both commonalities and differences among



Monomethyl phthalate [R_1: CH_3]
Monoethyl phthalate [R_1: CH_2CH_3]
Monoisobutyl phthalate [R_1: $CH_2CH(CH_3)_2$]
Mono-*n*-butyl phthalate [R_1: $(CH_2)_3CH_3$]
Mono-*n*-pentyl phthalate [R_1: $(CH_2)_4CH_3$]
Mono-*n*-hexyl phthalate [R_1: $(CH_2)_5CH_3$]
Monocyclohexyl phthalate [R_1: C_6H_{11}]
Monobenzyl phthalate [R_1: C_7H_7]
Mono(2-ethyl-5-carboxypentyl) phthalate [R_1:$CH_2CH(C_2H_5)C_3H_6COOH$]
Monoisopropyl phthalate [R_1: $CH(CH_3)_2$] (internal standard)

Fig. 1 Chemical structures of urinary phthalate metabolites [9]

phthalates in their uses and thus sources of exposure. Following exposure and uptake, phthalates are rapidly metabolized and excreted in urine and feces. They typically undergo phase I hydrolysis followed by phase II conjugation in human body [8]. In phase I, the phthalate diesters are hydrolyzed into monoester metabolites by lipases and esterases in the intestinal epithelium, live, blood, or other tissues. The monoester metabolites then undergo phase II biotransformation catalyzed by uridine 5′-diphosphate glucuronosyltransferases (UGTs) to form glucuronide-conjugated monoesters that are excreted in urine. Thus, detecting urinary biomarkers has been the most common method for assessing phthalate exposure in environmental epidemiology studies to date.

Exposure biomarkers have the advantage of being a measure of internal dose, which could account for all routes of exposure. This is desirable in epidemiology studies of chemicals with multiple exposure pathways. Many different types of biospecimens have been used to assess the environmental chemical exposure. Phthalates have been measured in urine and blood (serum, plasma or whole blood) as well as saliva, semen, breast milk, swear, amniotic fluid, and umbilical cord blood. However, measured concentrations in urine are normally much higher than the concentrations in other specimens. Measuring phthalates in urine offers many advantages over measuring the diesters or their metabolites in blood, which include (1) ease of sample collection; (2) larger sample volume; (3) higher concentrations of the metabolites; and (4) reduced potential for contamination by the parent diester and subsequent formation of metabolites by enzymes [10]. Moreover, for some phthalates, such as DEHP, the monoester metabolites are thought to be more biologically active than the parent diesters.

Moderate to strong correlations between urine and serum concentrations have been reported for the secondary metabolites of DEHP and for the primary metabolite of DEP [11, 12]. Oral dosing studies in human have shown that DEHP concentrations peak in serum within approximately 2 h of exposure and decline rapidly [13]. Reflexively, urinary metabolite concentrations rise, with almost all of orally administered dose excreted within 24 h after exposure. At the individual level, significant correlations between phthalate metabolite concentrations in serum and urine may not be expected, but moderate to strong correlations in these two matrices are plausible at the population level.

Phthalate-related epidemiological studies normally examine a large number of metabolites due to the multi-analyte nature of the most widely used assays in measuring the metabolites of phthalate. Various approaches have been proposed to reduce the number of exposure variables in these diseases. These approaches include (1) summing phthalate metabolites of “low molecular weight” and “high molecular weight”; (2) summing metabolites of the same parent compound; and (3) summing all measured metabolites regardless of parent chemicals. Though the application of these approaches could successfully reduce the number of statistical comparisons involved, they also have some obvious disadvantages. The approaches above rely greatly on an assumption that the chemicals or metabolites being summed have similar biological activities and targets. Nevertheless, this may not be plausible, even if the metabolites are summarized from the same parent phthalate while the

monoester itself is the most biologically active form. Similarly, when more nuanced approaches are applied to weight the toxicities of single metabolites based on combinations of the empirical data, there is an assumption that the same basis being used for the weightings represents the most sensitive endpoint for each individual phthalate.

Measuring phthalate metabolites in urine is currently the most common method of assessing exposure in epidemiological studies. The advantage of this approach is the wide availability of sensitive analytical methods, where most phthalate metabolites represent the specificity of a particular parent chemical, and the long biological half-life of monoester metabolites compared to the respective phthalate diester typically measured in serum. The possibility of contamination of enzymatic activity is reduced compared to the measurement of parent chemical or metabolites in other biological fluids. Limitations of this approach include consideration of half-lives on the order or hour, consideration of urine dilution (and uncertainty of the most appropriate method for urine dilution), and metabolite concentrations measured in urine represent only an approximation for the dose at the target site. Moreover, individual biomarker measurements do not provide information on the exposure pathways required for risk management. Studies that assess sensitivity and specificity have shown that a single measurement may possibly be able to reasonably predict long-term exposure categories (i.e., months, with limited data for years). However, collecting additional samples will improve sensitivity, and researchers should strive to conduct studies that collect multiple samples for measuring phthalate metabolites in the exposure window that is most relevant to the results of interest.

In summary, the measurement of phthalate metabolite concentrations in urine can be used as an effective method for estimating exposure in epidemiological studies. However, the advantages and limitations of this approach need to be carefully considered when interpreting the results of these studies.

2. **Bisphenol A**

Bisphenol A (BPA), a common industrial chemical synthesized by condensation of two phenol groups and one acetone molecule, is often used in food and beverage packaging, medical equipment, electronics, flame retardants, adhesive, building materials, automobiles, and paper coatings [14]. The first commercial production of BPA was in the United States in 1975. In the following 60 years, BPA becomes ubiquitous in the environment, thus increasing the likelihood for human exposure.

Since a large number of studies have reported the estrogenic properties of BPA [15], it is described as an endocrine disruptor chemical (EDC). BPA is capable of binding and activating human estrogen receptor, but with a capacity of 1000–5000 times less than the endogenous 17-β-estradiol. BPA has been shown to interact with other endocrine receptors including thyroid hormone receptors and peroxisome proliferator-activated receptor gamma [16]. BPA is classified as a reproductive toxic substance of category 3 as an alarming substance for human fertility.

Biomonitoring of BPA in various human matrices has been reviewed previously [17]. Urine is traditionally the most preferred matrix because BPA is extensively bound by glucuronidation and excreted in the urine, and sampling is minimally

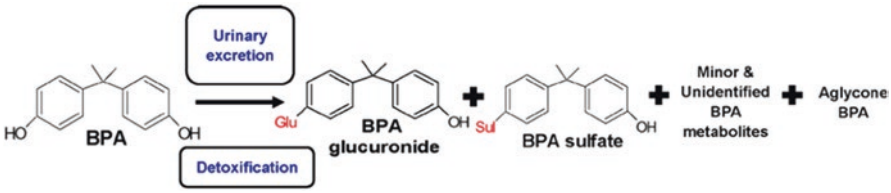


Fig. 2 BPA and its conjugates [20]

invasive. However, given the short half-life of BPA in human body(~6 h), the observed levels in urine could only reflect recent exposure, limiting its utility as biomarker [18]. Current analytical methods determine the total BPA concentrations after enzymatic hydrolysis preferably at 37 °C for a few hours and overnight in some specific situations. Typically, only β-glucuronidase is used in the de-conjugation step since BPA is predominantly present in the form of a glucuronide conjugate, while few have additionally used sulfatase enzyme for the release of BPA from the sulfate conjugate that occurs as a very minor fraction. In addition to the glucuronide form, the sulfatase will also be uncoupled if the β-glucuronidase is derived from *Helix pomatia*-H1 compared to *Escherichiacoli*-K12 [19]. Some of the problems leading to inaccurate or underestimated measurements may be due to suboptimal conditions of total BPA that arose from insufficient enzyme concentrations, improper selection of enzymes, incomplete deconjugation, unfavorable hydrolysis conditions and overall suboptimal de-conjugation. The structures of BPA and its conjugates are illustrated in Fig. 2.

Glucuronide and sulfate conjugates are the most common metabolites of xenobiotics and exogenous chemicals that undergo phase II metabolism in humans as a detoxification measure. Such conjugates are preferably detected and measured using LC-MS based methods. Several studies have reported the simultaneous determination of aglycone BPA and its major conjugates BPAG, BPAs and BPADS. Until recently, research was limited by the lack of custom synthesis of BPA conjugates and the commercialization of the respective labeled internal standards. Although some studies have reported the analysis of conjugated BPA in bio-specimens, the information was derived from taking the difference between total and free BPA rather than directly measuring BPAG and BPAS. Previous studies used urine [21], plasma [22], serum, and cord serum [23] to measure BPA and its conjugates. Extraction and satisfactory recovery of BPA and its conjugates from biological samples is critical, followed by their optimal separation from matrix ions, which produces optimal ionization, detection, and quantification through the MS platform.

Most studies used liquid chromatography to separate aglycone BPA and its conjugates. All reported methods used a gradient elution of concentrated and unaltered solvents made with acetonitrile or methanol [22]. The run length and retention times of aglycone BPA and its conjugates depend on the chromatographic conditions used in these studies. Run time ranged between <5.0 min and 22.0 min. The BPA conjugate elutes before the aglycone BPA and therefore has a shorter retention time

compared to the latter. Electrospray ionization (ESI) mode was the only optimized ionization mode. The presence of a phenolic hydroxyl group in the aglycone BPA and its conjugate promotes efficient ionization in the negative electrospray ionization mode and is therefore preferred. In the ESI negative mode, most studies used the following multiple reaction monitoring mass conversions to accurately detect and quantify BPA: m/z 227–2012; BPAG: m/z 403–113; BPAS: m/z 307–227; and BPADS: m/z 387–307. Isotope-labeled internal standards are used in the initial stages of sample preparation to ensure that quantification is not significantly affected by matrix effects.

Total BPA measurement in urine is a reliable measure only when external contamination is controlled. Since the introduction of BPA from an external source not only increases the aglycone but also increases the total BPA level, measuring either one is not necessarily a reliable biomarker of exposure to BPA. However, BPAG and BPAS quantification are gaining consideration and value because the levels are not affected by the general sample processing procedures either during collection or analyses. BPA and its conjugates in human matrices are primarily quantified as an integral part of method development, and very few studies have applied this method to biological monitoring of human exposure to BPA. However, BPAG and BPAS quantification are gaining consideration and value because the levels are also not significantly affected by the general sample processing procedures during collection. BPA and its conjugates in human matrices are primarily quantified as an integral part of method development, and very few studies have applied this approach for biomonitoring of human exposures to BPA. The specificity of BPAG is similar to aglycone BPA in exposure assessment studies [24–26]; however, the frequency of detection of BPAG in urine is almost always greater than aglycone BPA. Among the populations studied, BPAG was measured in almost all human matrices and was measured at higher detection frequencies and concentrations than aglycone BPA.

Urine BPAG concentrations account for 90–95% of total BPA, while aglycone BPA accounts for only 1–2% [27]. In contrast, one study reported that BPAG and aglycone BPA accounted for 57% and 32% of total BPA in urine [21]. The lower proportion of BPAG in this study was attributed to the possible external contamination of BA samples and the underestimation of BPAG using inappropriate internal standards (labeled BPA instead of BPAG). In the case of umbilical cord serum, BPAS is frequently detected and is a major fraction of the total BPA of 45%, followed by 36% aglycone BPA and 19% BPAG [28]. These conversion factors are very important when directly measuring BPAG and BPAS before comparing BPAG and BPAS with BPA exposure data.

The main limitation of using BPAG and BPAS as additional biomarkers for exposure to BPA is that if the urine sample is stored at room temperature, the conjugated form can degrade within a day or two [29]. This leads to underestimation of BPA exposure when the individual conjugates are analyzed and measured directly. BPAG is stable at −80 ° C for a total duration of 28 days and is stable in at least three freeze and thaw cycles [30]. While urine samples are properly stored, they may be exposed to ambient temperatures for a period of time during collection, handling, and transportation, which may potentially compromise the utility of these

surrogate biomarkers. When total BPA is used as a biomarker, filled blanks and reagent blanks are used as quality control methods to control background contamination of BPA, but no such measurements can be used to estimate degradation of urine samples between sample collection and pre-analysis. Other potential limitations of the method include: (1) lack of documented statistically significant, positive, and linear correlations between BPAG and total BPA; and (2) unknown and unrecognized outside BPAG and BPAS BPA conjugates are unmonitored and unmeasured.

Although BPA conjugate measurements are possible due to recent advances in analytical methods, there are some limitations and challenges that need to be overcome before considering their application in human biomonitoring research. If successful, analysis of BPA conjugates as additional biomarkers may help to better understand metabolic and distributional differences in life stages, inter-individual differences, and potential health effects of biologically active forms.

3. **PBDEs**

Since the 1970s, polybrominated diphenyl ethers (PBDEs) have been produced due to the ban on the use of previously used flame retardants. They are used as additives in a variety of polymers and are found in a variety of consumer products, including furniture, electronics, and automotive parts. PBDEs are environmental contaminants with persistent and bioaccumulative properties. Studies have detected PBDEs in human tissue samples such as placenta, adipose tissue, breast milk, and blood. The molecular structure of PBDEs is shown in Fig. 3.

Human exposure to PBDEs is thought to result from dietary sources, including fish, fatty foods, and breast milk. However, oral ingestion from dust and leachates may be a larger source, particularly for children. Once absorbed, PBDEs rapidly distribute into body fat. The metabolism and elimination of PBDEs in humans has not been well characterized. Some studies have shown that the elimination half-life of decabromodiphenyl ether is 11–18 days and the half-life of octabromodiphenyl ether homologs is 37–91 days [31]. In animals, PBDE elimination occurs primarily through fecal excretion, with decabromodiphenyl ether being eliminated more rapidly than other less brominated PBDEs.

Polybrominated diphenyl ether homologs for biological monitoring typically include three bromines (BDE-17, BDE-28), four bromines (BDE-47, BDE-66), five bromines (BDE-85, BDE-99, BDE-100), hexabromo (BDE-153, BDE-154), and heptabromo (BDE-183). Due to its low lipid content, low organic contaminant

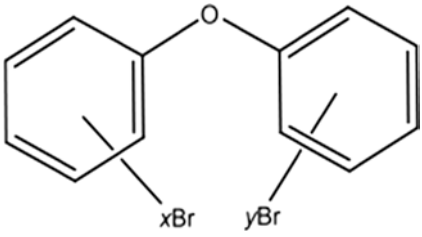


Fig. 3 The molecular structure of PBDEs ($x + y = 1$~10)

concentrations (usually below 1 ng/g), aqueous properties and high concentrations of hydrophobic proteins, many laboratories have demonstrated that blood analysis is difficult. Improved extraction efficiency, cleaning methods, and instrument sensitivity are needed to solve these problems.

Historically, liquid–liquid extraction (LLE) methods have been used most frequently to extract organohalogen contaminants like PBDEs from blood [32]. LLE allows for the extraction of large volumes of different matrices (whole blood, plasma, or serum) at relatively low extraction costs. However, this method is labor-intensive and does not allow for high throughput analysis. Solid-phase extraction has recently become a common method of blood extraction, and automated SPE has proven to be useful for large-scale monitoring projects using human blood samples. Microwave-assisted extraction is an alternative to LLE and SPE, but organic pollutants from blood have never been explored before. Cavity-dispersed microwave-assisted extraction (MAE) is widely used to analyze heavy metals in biological samples and to extract organochlorine contaminants from solid biological tissues and should be suitable for extracting PBDEs from blood matrices. In MAE, multiple samples are placed in an oven where microwave energy is dispersed throughout the oven cavity, while a focused microwave (FME) system applies all of the microwave energy to a single sample in a smaller over cavity, which leads to higher overall energy transfer to the sample compared to MAE. Among all the above methods, FME was found to be the most reliable one for extracting PBDEs from serum. It may result in the highest IS recovery and low to moderate variability without affecting the measured PBDE concentration. All methods except FME have quantitative problems with PBDEs. Furthermore, the highly reproducible concentrated microwave energy from this method may be the reason for its optimum performance. In addition, automation of extraction procedures, reduced solvent requirements and convenience, and reduced labor in the use of disposable glass extraction vessels may also be logistical reasons [33].

Measuring PBDEs in breast milk is vital to study the adverse health effect of early life exposure. PBDEs are lipophilic and can easily accumulate in breast milk. Hence, a major source of exposure to PBDEs for breastfed infants in early postnatal life is through the breast milk. Lin et al. introduced a tandem SPE cleanup method based on sulfuric acid silica cartridge coupled with basic alumina cartridge to measure PBDEs in human milk sample [34]. In contrast to the automated sample clean-up system (ASCS), SPE method presented comparable specificity with highly reduced consumption of solvents which results in much lower background in the procedural blank, reduced time, and enhanced sample pretreatment throughput. However, no present study has compared the performance of SPE method and FME method in extracting PBDEs from human breast milk, which warrants further investigation.

In addition, human hair and nails are good examples of noninvasive matrices. These matrices are easy and inexpensive to access and collect compared to other biological samples and are easy to transport and store. In addition, these matrices are stable with low or no active metabolic activities. Once the chemicals are incorporated into the keratinous tissue of hair or nails, the levels remain relatively uncharged because both of them are slowly growing tissues. Therefore, they provide

the opportunity to measure exposures in a time window from 1 week to 1 month, which could reflect past and present exposures. The correlations of the concentrations of PBDEs in serum with those in hair/nails were tested previously, and the results showed that significant positive correlations were observed for the levels of individual BDE congeners (e.g., BDE-28, -47, -99, -100, and -154). However, no significant associations between the levels of BDE-209, TBB, or TBPH in hair, fingernail, and toenail versus serum were observed. Rapid metabolism and elimination are a plausible reason. Due to the metabolism in the body, it is unlikely that BDE-209 or TBPH can be transported through the body and reach hair and nails. The high levels of them in hair and nail samples may come from external sources such as absorption from air and dust [35].

Though serum PBDEs levels could better reflect human exposure, the use of noninvasive matrices, such as on hair and nails, is highlighted due to the easy accessibility and collectability. Moreover, PBDEs in breast milk is also an important indicator to analyze the adverse effect of early life exposure. The quantification of PBDE species with short half-life like BDE-2019 still needs to be further investigated.

3 Nontargeted Approaches and Exposome

Only a few hundred chemicals are routinely assessable through targeted methods. The exposomic approaches are critical to understanding the thousands of chemicals which people are exposed to everyday through direct chemical exposure consequences (e.g., cortisol levels due to exposure stress or noise). Through nontargeted biomonitoring approaches like high-resolution metabolomics, thousands of compounds can be monitored with a relatively small amount of biological specimen (about 100 μL) and for the cost of a single traditional biomonitoring analysis of around 10 target chemicals [5].

Nontargeted analyses of small molecules or macromolecular adducts could be conducted in various kinds of biospecimens. Such methods compare features of thousands of chemical features which are similar to ions with a given mass-to-charge ratio and a specified retention time in traditional biomonitoring process. Blood, urine, and other matrices are well suited for exposome-wide association studies (EWAS). Indeed, untargeted analyses performed using the current generation of liquid chromatography-high resolution mass spectrometers can detect over 30,000 small-molecule features [36] and over 100 human serum albumin (HSA) adducts of reactive electrophilic chemicals at the nucleophilic locus Cys34 [37]. Dealing with the rich datasets derived from untargeted analyses of multiple biospecimens offers a solution for discovering harmful exposures that have escaped scrutiny so far. It is also vital to know that full annotation of molecular features is a necessary part for case–control comparisons while LC-HRMS signatures are available. Archived biospecimens from well-designed cohort studies already exist. With continued advancement in untargeted analyses, there is potential to make significant advances in human health through uncovering unknown exposures [38, 39].

However, challenges exist with detecting chemicals, especially for those at low levels. It is also hard to define reference doses of "normal" exposure and linking these measures to active intervention approaches. Because blood xenobiotics concentrations tend to be greatly lower than the chemicals derived from drugs, food, or endogenous sources, nontargeted analyses of xenobiotics are not as efficient and reliable as those of ingested and endogenous sources for detecting many exposures of interest [40]. To determine the health impacts of these exposures, it is necessary to develop semi-targeted or multiplexed methods that increase the signals of exogenous molecules relative to those of endogenous origin. Analyzing suspected chemicals of concern (or suspect screening) can be prioritized through measuring groups of chemicals with known biological effects, but specific assumptions about toxicological pathways cannot be determined. Exposomic biomonitoring, including untargeted and suspected chemical analyses, has offered extraordinary potential for increased understanding of complicated chemical exposures.

When using the exposome approaches, special attention must be paid to which matrices can be practically collected and which matrices are associated with the assessment of chemical. In general, the least invasive specimens, such as blood and urine, are the preferred matrix in which the chemicals could be conveniently collected [41]. Although most exposure analyses are performed with urine or blood samples as they can be easily collected, there are other sample types which are also valuable in exposome interrogation. For example, saliva can be collected from school-age children and adults but is also a problematic matrix to collect from newborns because of choking dangers associated with the collection devices. Even if the matrix can be easily collected without invasion, it may not contain the target chemical or potential chemicals for many reasons, such as protein binding that prevent their secretion [42]. In addition, in nonsterile saliva, the contribution of the oral microbiome can affect the composition of the analytes to be tested. Buccal and nasal swabs are also used to assess the biological consequences of external exposure. In those samples, DNA, mRNA, and their adducts have been the main focus to date [43], but these sample types could also be significantly affected by the presence of powerful microbial communities that can affect the composition of exposed components.

When selecting samples for exposome-type research, an important consideration is the expected presence of a particular chemical in the harvested sample. Because chemicals may exhibit unexpected pharmacodynamics and biotransformation, it could be essential to collect multiple sample types from each individual to define the exposome. Blood circulates throughout the body, so its assessment has an advantage because it has been exposed to countless ways in which environmental chemicals enter the body. However, it is known that some analytes could specifically accumulate in specific tissues. Therefore, a broad-spectrum assessment of samples collected from multiple patient samples will provide the best insight into exposome.

The identification of unknown chemicals remains a major challenge for understanding the exposome. Research should try to prioritize the development of methods for determining relevant exposures and identify the sources of specific chemical characteristics. By correlating changes in microbiome, metabolomes, proteomics, etc. with unknown analytes, we can begin to determine the profile of the unknown

toxic exposure and its consequences. In addition, biomonitoring techniques that can assess changes in cell composition or cell developmental capacity may identify the risk of subsequent health conditions such as cancer and neurodegenerative diseases. Linking unknown exposure to potential disease outcomes can further support understanding the resource inputs required to accumulate lifetime exposure.

Exposome analyses normally based on high-throughput advanced mass spectrometry technology. Analyzing bio-matrices to acquire the totality of exposure to organic pollutants is a small part of the assessment of the large and complex internal chemical environment consisting of exogenous reactions and a component for expanding human exposome [44].

Advanced high-resolution mass spectrometers (MS) allow expanded metabolic detection, leading to the exploration of a wider, untargeted chemical space in the exposome [45]. Andra et al. proposed a workflow for the analysis using the analysis of small molecule and metabolites as an example. This workflow including identification, quantification, and integration of the exposome into health studies (Fig. 4). The generic approach has two consecutive aspects: First, using nontargeted or "discovery" methods that employ high resolution mass spectrometry detection following liquid chromatography (LC) or gas chromatography (GC) separation to generate one large mass spectrometry data set; second, large-scale fragmentation of organic compounds and biomolecules which could be affected by environmental exposures. This approach could be combined with data mining via a series of statistical approaches and are useful in identifying markers that can then lead to targeted analytes.

The annotation of unknown chemical species could be extremely time-consuming and therefore only done on specific features. Through the joint efforts of many research groups to identify, catalog and disseminate information related to newly discovered small molecules, the limitations on chemical annotations can best be overcome. In addition, continuing to focus on bioinformatics techniques to extract information about important chemical characteristics will allow for semi-targeted methods for unknown and low abundance chemicals.

The omics technique has the potential to discover unknown compounds. Through the continuous advancement of mass spectrometry, low abundance chemicals can be located and characterized. By comprehensive coverage of the metabolome, the reference metabolic profile combined with health outcome data will provide a baseline for identifying unknown analytes with health relevance. Identifying and cataloging unknown analytes through cross-laboratory efforts will be a real task in advancing the exposome.

The application of nontargeted metabolomics to identify environmental exposures associated with human health is facing some unique challenges. The largest reference databased for metabolomics including the Metabolite and Tandem MS Database (METLIN) and the Human Metabolome Database (HMDB). Both databases focus on naturally occurring metabolites. The number of compounds in METLIN and HMDB that may be relevant to exposure studies has not been well estimated yet. The number of databases available for metabolomics is continuously expanding and has unique utility based on research questions. A broader discussion of metabolomics database resources can be made. To facilitate large-scale exposure

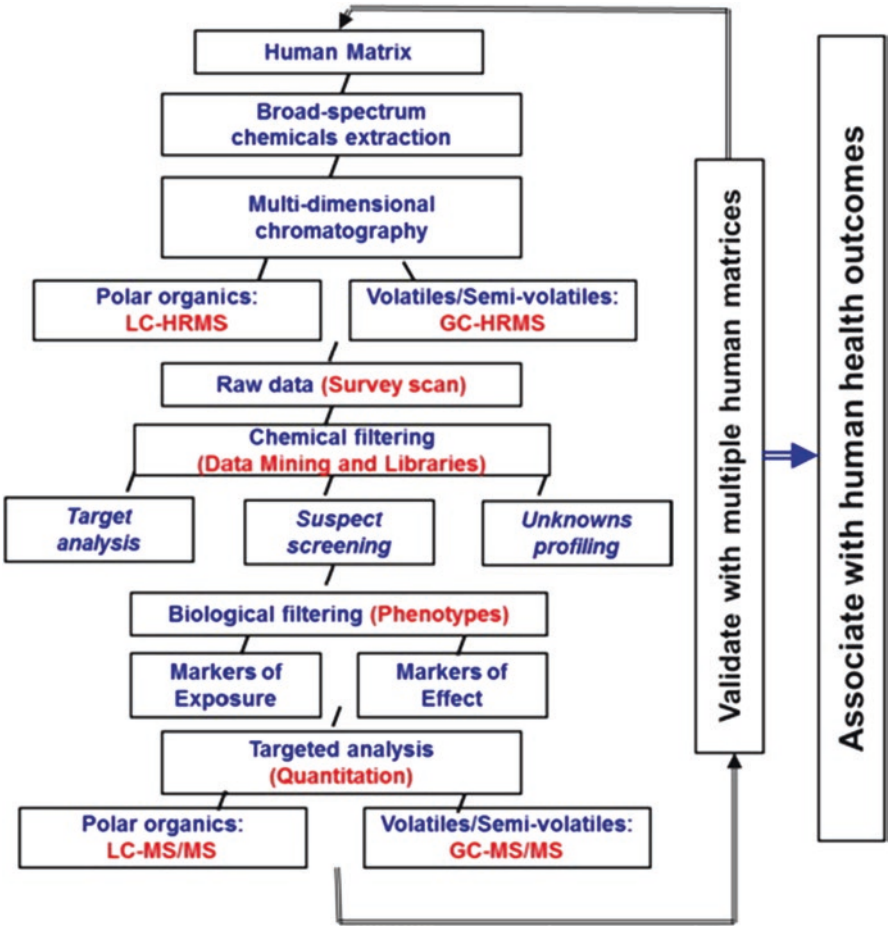


Fig. 4 An overview of applying high resolution mass spectrometry in human biomonitoring studies [46]

omics research, the field can benefit from owning a database or having a database search function dedicated to environmentally exposed chemicals.

Manrai et al. described that the human exposome data structure is a high-dimensional set of highly heterogeneous exposure variables that may change during repeated sampling in an individual's lifetime (Fig. 5a) [47]. Time-dependent and high-throughput genomic-scale phenotypic data types such as gene expression, protein expression, and metabolomics data are structurally similar.

However, there still a gap in assaying to operationalize an exposome-wide measurement platform. There are still signifijcant challenges in the field of metabolomics, such as determining the exact chemical identity or structure of an analyte in human tissue detected from mass spectrometry. In addition, another important challenge is the data integration across external and internal exposed domains. For

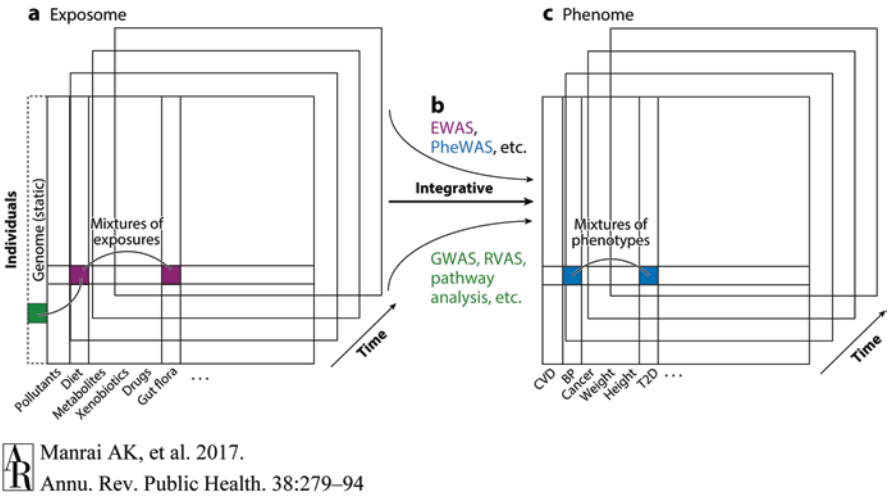


Fig. 5 Data analyses in high-throughput exposomic studies [47]

example, how does sensor and physical measurements or socio-demographic factors at the individual level affect the results? How the distribution of internal exposure indicators affects the distribution of internal exposure indicators? Data integration across these domains is typically achieved by merging across space–time coordinates, a resource-intensive but often straightforward database task. Integrating the multidimensional and vertical data streams that appear in external and internal exposure measurements remains a huge challenge. Methods such as Specification Correlation Analysis or Graphical Minimum Angle Regression (Graphical LASSO) [48] techniques can enable investigators to map on large dataset to another, but methods are missing to consider longitudinal data. Computational methods enjoying a resurgence in the data science community, such as neural networks, may also be harnessed to assimilate data over different dimensions [49].

Information to support the interpretation of primary findings will require the continued development of ontologies and databases that are relevant to the exposome. Two established databases will help to develop standards for naming and toxicology knowledge, namely the Toxic Exposome Database (T3DB, http://www.t3db.ca) and the Comparative Toxico-genomics Database (CTD, http://ctdbase.org). T3DB currently provides information on more than 3600 compounds and over 2000 targets, expression datasets of more than 15,000 genes, and extensive information on chemical concentrations in biological fluids and reference chemical spectroscopy data. Software libraries such as GitHub (http://github.com), development tools such as WebProtege, and portals such as Bioportal can implement public and open source development of ontology. Discovery research in contact with humans is a big data analytics integration challenge involving statistics, computer science, biomedical, and public health. Further investigations need to make full use of the information in the high-dimensional exposure data.

4 Summary

In this chapter, we briefly discussed the current status of the application of both targeted approaches and nontargeted exposomic methods in the exposure assessment of chemical pollutants. Traditional biomonitoring studies typically focused on a specific group of interest chemicals such as phthalates, BPAs, and PBDEs. Recent studies tended to use noninvasive or less-invasive bio-matrices which could be accessible in sufficient amounts for the analysis and do not pose a health risk for the donor. There is not an ideal matrix for universal situations, but depending on the target chemical, toxicokinetic of the chemical, LODs, available amounts, and so on. After exposure, chemicals leave traces in human organism. For some compounds, this trace can be easily detected but not for others. Therefore, new exposure or effect biomarkers must be continuously researched and improved to detect this kind of traces. The development of group biomarkers is particularly important for targeted biomonitoring studies which are designed for those purposes.

At the current stage, targeted approaches are indispensable due to the huge implemental and statistical challenges that nontargeted exposomic studies are facing. The exposome concept is both a challenge and a potential driver for environmental health studies. The exposome is still in its infancy. Many technical and statistical challenges remain unsolved. In the case of assessment, hundreds of time-varying exposures need to be accurately assessed. Nevertheless, increasing the number of exposures assessed should not be done at the cost of increased misclassification. Estimating the association between a large amount of exposures and health could lead to statistical problems, including the increasing false discovery rate as well as the difficulty in efficiently untangling the exposure truly affecting the health outcome as they are significantly correlated with each other. These challenges should not hinder research that relies on the exposome methods, and the ongoing projects will hopefully allow advancement.

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