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INVITED REVIEW

Reproductive Health

Human biological monitoring of suspected endocrine-disrupting compounds

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Endocrine-disrupting compounds are exogenous agents that interfere with the natural hormones of the body. Human biological monitoring is a powerful method for monitoring exposure to endocrine disrupting compounds. In this review, we describe human biological monitoring systems for different groups of endocrine disrupting compounds, polychlorinated biphenyls, brominated flame retardants, phthalates, alkylphenols, pesticides, metals, perfluronated compounds, parabens, ultraviolet filters, and organic solvents. The aspects discussed are origin to exposure, metabolism, matrices to analyse, analytical determination methods, determinants, and time trends.

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INTRODUCTION

Human biological monitoring (HBM) is a method of obtaining information regarding (i) exposure, (ii) effects or other responses, (iii) susceptibilities, or (iv) diseases. The information is obtained by analyzing compounds in human biological matrices, mainly blood or urine; however, other matrices are also used such as saliva, amniotic fluid, hair and semen, and so on. In this review, we will focus only on the HBM of exposure, where the compound itself or a metabolite is analyzed. HBM of exposure has many advantages compared with environmental monitoring, that is, to analyse the substances before becoming exposed through food, water, surfaces, or air. HBM measures a sum of the total internal exposure from all exposure routes. Furthermore, it is often easier to collect many samples using HBM than environmental monitoring. In addition, if the toxic mechanism of the compound is known, it is possible to take metabolic differences into account using HBM. It has been argued that the analytical methods used for HBM are difficult to develop due to the complicated biological matrices; however, new, sophisticated, analytical equipment has to a large extent facilitated the development of such methods.

For the past 5 decades, an increasing trend of awareness regarding xenobiotics and their endocrine-disrupting capabilities can be noted. An "endocrine-disrupting compound (EDC) is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior," as defined by a USA EPA 1997 report. HBM has been beneficial in creating background information of endocrine disrupting compounds and is extensively applied to epidemiological studies of diseases due to occupational and environmental exposures. HBM serves as the basis for setting reference values, health-risk assessment, and management. The careful choice of biomarkers is required, keeping in mind their toxicokinetic stability, specificity, and reliability. Detailed information of guidance on biomonitoring, HBM requirements, study design, health-risk assessment, ethical requirements, and many more aspects can be found in the referred articles.[1–4]

The American Conference of Governmental Industrial Hygienists and the Deutsche Forshungsgemeinschaft are important organisations involved in setting of HBM reference values for occupational and environmental health. Several countries routinely perform large-scale general population surveys such as the National Health and Nutrition Examination Survey in the USA, the German Environmental Surveys in Germany, and the relatively newly formed consortium to perform human biomonitoring on a European scale in Europe. In this context, the importance of interlaboratory control programs should be emphasized to allow for the comparison of the results of different monitoring programs.

HBM has produced vital information that has led to control of many suspected EDCs, such as pesticides, heavy metals, polychlorinated biphenyls (PCBs), polyfluorinated compounds (PFCs), and brominated flame retardants (BFRs), which have concentrations that have shown declining trends in the general population globally. HBM data are usually interpreted by comparison with these reference values set by the responsible organizations. In the case of an absence of reference values, the results are compared with recommendations in the scientific literature.[3] For example, the German Human Biomonitoring Commission recently published an update on the reference values and HBM values for a number of environmental pollutants in urine and blood matrices.[5]

This article focuses on groups of selected xenobiotic compounds that are suspected EDCs. An attempt has been made to review the

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information regarding the exposure of EDCs to humans, commonly followed analytical methods for their analysis and studies performed for monitoring determinants of these EDCs in humans.

POLYCHLORINATED BIPHENYLS

PCBs have been used in industrial oils, plasticisers, pesticides and, as coolants, in electrical transformers. PCBs comprise 209 congeners and may differ with respect to their uptake and metabolism. They can be broadly classified into two groups: the coplanar or dioxin-like congeners and the non-coplanar or non-dioxin-like congeners. Generally, non-dioxin-like PCBs are more commonly found but less toxic than the dioxin-like PCBs. In spite of being banned in the late 1970s, these chemicals can still be found widely in the environment. Global observations have suggested that the major sources of PCB emissions are in developed countries.[6] The main route of human exposure to PCBs, as many other contaminants, is food, especially fatty fish and meat.

The determination of PCB congeners has been performed in serum,[7] plasma,[8] breast milk,[9] and hair,[10] mainly by gas chromatography-mass spectrometry (GC-MS) or GC-electron capture detection. Sample preparation techniques such as on-column degradation of lipids in serum samples have been used in many studies.[7,11] Another method reported PCB extraction from plasma samples by *n*-hexane followed by clean up on a silica-gel column.[8] Breast milk samples were subjected to mixed organic solvents extraction, followed by fractionation on activated carbon and further purified by adsorption chromatography on alumina.[9] A detailed overview of several other analytical techniques and sample extraction methods can be found in the literature.[12] Small amounts of biobanked samples can be accessed using analytical methods that only require a few hundred micro liters of sample. Concern has been expressed over the validity of PCB-153 serum levels without lipid adjustment of the values. However, high correlations between the fresh-weight and the lipid-adjusted values have recently been reported, alleviating the concern.[13]

The non-dioxin-like PCB congeners CB-138, CB-153, CB-170, and CB-180 have high correlations to other PCBs and known health effects; hence, they are usually determined to measure exposure in humans.[14] Many epidemiological studies have related fish-consuming habits to elevated PCB concentrations, such as in the North American Inuit population[15] and Greenland Inuits.[16] A similar trend was observed in various Swedish fishermen cohort studies.[17–21] Time-trend studies have revealed that PCB concentrations have decreased over the past decade from 1991 to 2001.[22] A rapid decline of CB-153 was also observed in young Swedish males.[23] Studies have demonstrated that CB-153 concentrations increase with increasing age, showing the older population with a higher body burden of PCBs.[7,24] PCBs can easily pass from mother to fetus and the breast milk in turn may increase the body burden of the infants.[25] Studies in mothers of southern Sweden showed serum levels of CB-153 in the range 0.1–11.4 ng ml^{-1}.[26] Pregnant women from Greenland showed elevated CB-153 levels compared with expecting mothers of East Europe.[27,28] Interpopulation biomonitoring revealed that Greenland Inuits and Swedish fishermen population are highly exposed to PCBs through seafood intake compared with the representative Eastern European population.[19] A total of 34 different PCBs were ubiquitously found in tested USA populations.[14] A rising trend of PCB exposure in developing countries such as China is evident due to the growing number of e-waste recycling sites.[10] Various studies have been published in the past several years evaluating the magnitude of e-waste recycling sites in China.[29]

BROMINATED FLAME RETARDANTS

BFRs are organobromine compounds used in a variety of textiles, thermoplastics, electric and electronic goods, building materials and vehicles due to their effective flame-retardant property. The flame-retardant products contain polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls, and tetrabromobisphenol A (TBBPA), which are known to be persistent and bioaccumulative. PBDEs constitute approximately 25% of all flame retardants, with 209 possible congeners, and are commercially produced as pentaBDE, octaBDE, and decaBDE. PBDEs and polybrominated biphenyls were listed among the six controlled compounds under the restriction of hazardous substances, and a ban has been imposed on the production and use of pentaBDE and octaBDE since 2003.[30] These compounds are used as additives to the polymers and are not chemically bound. They, therefore, tend to leach out from the surface of the products. Restriction on BFRs has given rise to the production of novel BFRs: 1,2-bis (penta bromo diphenyl) ethane, commonly known as decabromodiphenyl ethane; 1,2-bis (2,4,6-tribromo phenoxy) ethane; 2-ethyl hexyl-2,3,4,5-tetrabromo benzoate; bis (2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate, tetrabromo bisphenol A-bis (2,3-dibromopropyl ether) (TBBPA-DBPE), and hexachloro cyclopentadienyl dibromocyclo octane.[31]

Exposure of the general population to BFRs can occur through contaminated food, indoor dust, or air. BFRs have been measured in human milk, blood and serum samples,[32] placenta[33] blood plasma[34], adipose tissue,[35] and frozen brain samples.[36] For pre- and postnatal exposure assessments, breast milk, cord blood, and maternal blood plasma are typically analysed.[35] BFRs have mostly been analyzed through GC-MS. GC methods usually involve several extraction and derivatization steps;[24,31,34,37,38] however, it is the preferred analytical technique due to its high specificity. Rarely, the liquid chromatography (LC)-MS/MS method has also been described for the analysis of TBBPA and hexabromocyclododecanes.[39] Sample extraction methods such as solid phase extraction (SPE),[37,38] liquid – liquid extraction,[40] or organic-solvent extraction and purification on silica-gel column[34] have been used and detected by GC-electron capture detection and GC-high-resolution MS.[38,41]

PBDE congeners BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154 have been ubiquitously reported in USA populations.[14] Studies have showed that fatty fish is an important pathway for BFR exposure.[35,42,43] Thus, specific populations, such as the Inuits of the Arctic region and fishermen populations, which have high consumption of seafood, are vulnerable to BFR exposure. Time-trend studies in the Western countries demonstrate increasing levels of BFRs in humans in the past 2 to 3 decades.[44] PBDE levels in USA populations are 10–100 times higher than most European countries; however, hexabromocyclododecanes levels observed in the USA were one to five times lower.[45] PBDE levels observed in the Swedish study are comparable or lower than those found in other studies performed in Europe.[24] PBDE levels observed in a maternal – fetal group in Japan were comparable to Sweden and lower than the USA.[40] Hexabromocyclododecanes levels in human milk in the UK are comparable to Norway and Canada, but higher than those found in Sweden, the USA, Russia, and Japan.[39] PBDEs measured in human tissues in China, especially in the occupationally exposed e-waste recycling workers, are in the intermediate to high range compared with the rest of the world.[32] The highest PBDE levels (613 ng g^{-1} lipid in serum and breast milk) were found in the population from the province producing PBDEs, with BDE-209 being the predominant

congener.[32] A consistent detection of PBDEs and polybrominated biphenyls in human tissues of e-waste recycling workers and residents from recycling sites in China has been reported.[10,46,47] Studies have shown that European children are being exposed to background levels of PBDEs. Breast milk and indoor dust are major sources of exposure for infants and toddlers to PBDEs. Infants exposure is estimated to be in the range of tens of ng kg^{-1} b.w. per day in Europe and hundreds of ng kg^{-1} b.w. per day in the USA[43] PBDEs and TBBPA have been reported to be present in French mothers, indicating a risk of BFR exposure to newborns.[48] Several studies of PBDEs and TBBPA in maternal and umbilical serum, maternal adipose tissue, breast milk, maternal blood, and umbilical cord blood have been reported for the exposure assessment of foetuses and newborns to BFRs.[40,48] Very few HBM studies have been conducted on the class of the novel BFRs. These BFRs have been detected in human milk and placenta samples in some studies, but most did not report any detection or very low levels in human samples.[31]

PHTHALATES

Phthalates are the most widely used plasticisers and have a varied range of applications from industrial to domestic household products. Apart from occupational exposure, the known sources of exposure to phthalates are air, water, dust, food, consumer products, and personal care products. Di (2-ethyl-hexyl) phthalate (DEHP) along with dibutyl phthalate, and benzyl butyl phthalate have been banned in children's toys by the European Union since 1999. However, many other phthalates are still commonly used, which make them one of the most widely found compounds in most of the matrices. Phthalates enter the human body through food, inhalation, or dermal uptake. Phthalates are not known to accumulate in the body but rapidly metabolise and are excreted in urine and faeces. They are metabolised to their respective monoesters, which in turn can undergo oxidation reactions and form conjugates with glucuronic acid.[49,50]

Several analytical methods are presented in the literature for the determination of phthalates, with GC-MS[51,52] and LC-MS/LC-MS-MS/tandem techniques[52–54] being the most popular. Sample preparation techniques such as liquid – liquid extraction, followed by pressurised liquid extraction[52] or manual or automated SPE[55,56] have been applied. Human urine,[54,55] blood,[56] semen,[54,56] breast milk,[52] and amniotic fluid[57] have been applied for HBM of phthalates in various studies. However, to avoid sample contamination, urine has been the preferred matrix for phthalate exposure assessment because of the lack of lipase activity and higher levels. Nevertheless, oxidative metabolites can be monitored in serum. Glucuronidase treatment is normally performed to release glucuronic acid conjugation.

Extensive reviews have been published regarding HBM of phthalates and metabolism routes in humans.[54,58,59] In adults, the highest levels are usually found for monoethyl phthalate,[54,60] while levels of monobenzyl phthalate appear to be low. The levels of mono-n-butyl phthalate are often between monoethyl phthalate and monobenzyl phthalate. The sum of DEHP metabolites is also rather high. The levels found are rather similar in different countries. Infants are considered prone to phthalate exposure due to their dependence on formulated food and breast milk, ingestion of dust, and so on, with the most concern expressed over those receiving intensive medical care. An investigation of the biomonitoring of phthalates in infant nutrition and reproductive health explains that infants should be considered at higher risk for DEHP exposure and the potential health hazards involved.[61] The phthalate metabolite levels in infants are at least as high as in adults.[62] Exposure to the fetus is considered to be of special relevance for the development of disease later in life. Thus, phthalate metabolite monitoring in pregnant mothers has been performed to monitor fetal exposure.[57,62] A more direct method is the analysis of amniotic fluid samples, which has been performed in 300 biobanked samples. The analysis showed detectable levels of DEHP metabolite: mono (2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) and DiNP metabolite: mono (4-methyl-7-carboxyheptyl) phthalate (7cx-MMeHP).[57] The DEHP metabolite levels decreased, while those of DiNP increased during the years 1980–1996.

In spite of current increased awareness, unfortunate incidents such as use of phthalates as a clouding agent in food and beverages has recently been reported,[63] posing a serious threat to the general population.

ALKYLPHENOLS

Bisphenol A (BPA) is a widespread alkylphenol used as a monomer in the production of polycarbonate plastic and epoxy resins used in the lining of metal cans and food containers, baby bottles, toys, plastic containers, water pipes, sports equipment, medical equipment, dental applications, and many other consumer products. 4-nonylphenol (NP) and 4-octylphenol (OP) are other important APs used as intermediates to produce alkylphenol ethoxylates. Alkylphenol ethoxylates are widely used in surfactants, emulsifiers, detergents, household cleaners, and personal care products and in paper, textile, leather, and agrochemical industries. BPA is one of the most highly produced EDCs on a global scale, with expected continued growth in future.[64] Humans are exposed to APs mainly through the intake of contaminated food and drinking water. Repetitive usage of polycarbonate products subjected to heat or varied pH conditions causes hydrolysis of ester bonds resulting in leaching of APs into foods and beverages. The current tolerable daily intake for BPA is 0.05 mg kg^{-1} b.w. per day. Recently, the European Commission made a significant decision to restrict the use of BPA in plastic baby bottles in the European Union.[65] However, infant food formulations and breast milk are still important BPA sources of concern. Glucuronidation and sulphation of APs is the main route of metabolism in humans, and the product is mostly excreted within 24 h. Unconjugated BPA and some other discovered metabolites are considered to be the biologically active form.[66]

For HBM, BPA has been measured in urine, serum, blood, breast milk, amniotic fluid, semen, placental tissue, follicular fluid, and umbilical cord blood.[67] NPs and OPs have been measured in plasma, urine, breast milk, and serum.[68] However, it should be emphasised that urine is preferable due to the higher levels and the high risk of contamination. Analytical techniques applied for measuring APs in humans are GC-MS, LC-MS/MS, and enzyme-linked immunosorbent assay. Detailed information regarding analytical techniques with respect to various biological matrices can be found in the referred article.[68] Although enzyme-linked immunosorbent assay is a cheaper option, its accuracy and precision is questionable. The sample is treated with the glucuronidase/sulphatase enzyme, and SPE extraction is often preferred. Other extraction techniques such as liquid – liquid extraction, stir bar sorptive extraction, and solid phase microextraction have also been applied.[68]

A number of reviews on HBM studies on BPA have been published in the past decade. Most exposure studies showed mean urinary BPA concentrations below 3 μg l^{-1}.[69] Although higher concentrations of BPA in blood and urine have been reported, these values were later considered inconsistent with other available data and toxicokinetic

studies.[69] A report suggests that BPA levels in developed countries are 400–2000 times below lifetime daily intake levels,[64] with a lack of information from developing countries.[67] The average urinary BPA reported in the USA (National Health and Nutrition Examination Survey) and Canada showed that children and youngsters had higher levels than adults.[14,70] Urinary BPA levels of American and European pregnant women ranged from 1 to 4.5 ng ml^{-1}.[71] BPA is known to pass the maternal-fetal placental barrier. Maternal and fetal serum, cord blood, amniotic fluid, and placental determination of BPA suggest risk of fetal exposure to BPA.[67] In addition, infants are considered particularly vulnerable to exposure due to the presence of BPA in breast milk. At higher risk are neonates receiving intensive medical care.[72] Lately, different views have been expressed over food being considered the only route of BPA exposure. A report showed lower estimates of BPA levels in food than from HBM data, indicating the significance of other exposure sources.[73]

NP and OP were detected in more than 50% of the urine samples obtained from the USA study population.[74,75] The consumption of foods such as seafood, fish oil, and cooking oils are suggested to cause dietary exposure to NPs and OPs.[76,77] NPs in tissue samples of Italy were reported to be two times higher than in Spanish and Finnish studies and three times higher than in Switzerland[78] and levels in Spain were comparable to other European studies.[79] Similar NP levels in Taiwan region were higher than most European and Japanese studies.[76] In general, there is a scarcity of data on HBM of NP and OP compared with the available data on BPA. More research needs to be directed on isomer determination, as many studies have determined exposure to technical mixtures of OPs and NPs.

PESTICIDES

Numerous pesticides are recognized for their endocrine-disrupting behavior. A compiled list of endocrine-disruptor pesticides has been recently published.[80] Several of these compounds have been banned in many countries; however, they can still be found in the environment due to their long half-lives or the continued usage by some countries. Pesticides can be broadly classified as organochlorines (OCs), organophosphates (OPs), carbamates, and pyrethroids. OCs, such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), endosulfan, heptachlor, and aldrin; OPs, such as malathion, chlorpyrifos, diazinon, dichlorvos, and fenitrothion; carbamates, such as aldicarb, carbaryl, and carbofuran; and pyrethroids, such as cypermethrin and deltamethrin are well known for their endocrine-disrupting properties. A significant focus has also been on the fact that chemicals can interact with other chemicals in a mixture, leading to toxicity change due to synergism and antagonism effects modifying the toxicokinetic properties of the compounds. The interactions of chemical mixtures at high doses are well known; however, there is a need to investigate the potential reactions of chemicals at low doses similar to current human exposure levels.[81]

Populations working in close contact with pesticides as well as the general public through the intake of contaminated food and drinks, domestic use, treatment of public places with pesticides for disease control or living in close proximities to agricultural fields bear the risk of potential exposure. Dermal exposure is considered a significant occupational exposure route for pesticide entry in humans. Human contact of pesticides can be monitored by measuring pesticides or the metabolite levels in blood, serum, urine, breast milk, and hair samples. Measurements have also been performed in umbilical cord blood and amniotic fluid samples to study foetal exposure to pesticides. OCs are usually determined in lipid-rich matrices as they are lipophilic and tend to accumulate in body fat. However, non-persistent pesticides are excreted rapidly and are hence determined as the parent compound and metabolites in urine.[82] OPs join with the cholinesterase enzyme and inhibit the activity of enzyme causing elevated levels of acetylcholine, which serves as indicator of presence of high levels of OPs. However, acetylcholine analysis in blood does not serve as a biomarker for low-level exposure for a longer time.[83,84] OPs and carbamates have similar working mechanism as acetylcholinesterase inhibitors. Polar compounds and metabolites are usually excreted through urine, and the less polar ones conjugate with glucuronide or sulphate to increase their polarity to facilitate excretion.[82] Hence, the samples are usually pretreated with glucuronide/sulphatase enzyme for deconjugation, and the sample is extracted by SPE. Some studies have described on-column degradation of lipids along with SPE for the determination of OCs in lipid-rich matrices.[7,11] The obtained concentration values are often adjusted for creatinine (in the case of urine) or lipids (in the case of blood or other lipid-rich matrices) concentrations. SPE is a widely applied sample extraction method because most biological samples are in the liquid phase, and the obtained limit of detection and recoveries are substantially better compared with other extraction methods.[85] Metabolites that tend to volatilize under GC conditions are determined using GC-MS. Most pesticide metabolites are less volatile and thermolabile; hence, LC-MS is the preferred technique for analysis.[85] Extensive reviews discussing pesticide analysis in biological samples are available in the literature.[82,85–87]

ORGANOCHLORINES

Heptachlor epoxide, oxychlordane, trans-nonachlor, 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p, p'-DDE), 1,1,1-trichloro -2,2-bis (4-chlorophenyl) ethane (p, p'-DDT), dieldrin, and mirex are the most commonly found OCs in humans, and p, p'-DDE is ubiquitous in the general population.[88] Among 19 regions/countries compared for *p, p'*-DDE and *p, p'*-DDT levels in human milk samples, Poland and Sweden showed the lowest concentrations, and China generally showed very high concentrations due to a late production ban and continued use of DDT in agriculture and disease control.[89] A review reported p, p'-DDE and p, p'-DDT concentrations in maternal and foetal blood to be lower than 500 ng^{-1} lipid, except in developing countries.[90] In a determination of other OCs in maternal and cord blood, β-HCH, hexachlorobenzene (HCB), trans-nonachlor and oxychlordane showed levels below 50 ng g^{-1} lipid, except Mexico.[90] In China, HCB levels in adipose tissue were comparable to levels in Japan and slightly higher than the USA; however, HCB levels in human milk samples were relatively higher than in the USA and other Asian countries.[91] A recent review documented a high exposure of Kenyan mothers to OCs especially DDT, dieldrin and lindane.[92] The mean DDT concentration in breast milk in South Africa was 15.83 mg kg^{-1}, and the mean DDE levels in breast milk and blood samples of Ghana were 490 μg kg^{-1} fat and 380 μg kg^{-1}, respectively.[92] Extensive studies on Swedish fisherman community showed exposure to p, p'-DDE due to contaminated fish consumption.[7,11,93,94] Large-scale epidemiologic studies conducted on pregnant women showed high serum levels of p, p'-DDE in Ukrainian mothers compared with Greenland Inuit mothers and Polish mothers.[27,28] HBM studies performed in Poland, Ukraine, Greenland, and Sweden revealed greater than 10-fold variations in the median serum p, p'-DDE concentration.[95] Compared with previous studies, a 15-fold decrease in serum DDT levels was observed in the USA population; however, detection occurred in nearly in all samples.[96] The age group 12–19 years had lower geometric mean serum levels than higher age groups. A similar trend was observed for heptachlor epoxide, transnonachlor, and oxychlordane.[96] Investigations in Sweden

also reported a continually decreasing trend, with 30% of samples having concentrations below the detection limit.[23]

ORGANOPHOSPHATES CARBAMATES, AND PYRETHROIDS

Metabolites of dialkylphosphates (DAPs) (dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate) are most commonly measured as OP metabolites.[97] The mean total DAP levels found in European studies were higher than those found in the USA.[98] Children in the USA generally showed higher levels of DAP metabolites than adults.[96,97] The 3,5,6-trichloro-2-pyridinol (TCPY) metabolite was detected in more than 90% of children's urine samples in a number of USA HBM studies.[99] Detectable levels of DAPs have been reported in the biological samples of children and pregnant women.[98] Ethylene bisdithiocarbamates, such as maneb, mancozeb, and ziram, are metabolised into very polar compounds, such as ethylenethiourea.[82] Commonly measured metabolites of carbamates-propoxur, carbaryl, and carbofuran are 2-isopropoxyphenol, 1-naphthol (1-NAP), and carbofuranphenol, respectively.[97] Permethrin, cyfluthrin, cypermethrin, and deltamethrin are commonly used pyrethroids. The long-term effects of pyrethroids are not known; however, neurological and endocrine-disrupting effects are the most commonly studied effects with regard to long-term exposure.[100] Pyrethroids are immediately hydrolysed in humans to form the metabolites 3-phenoxybenzoic acid (3-PBA), cis- and trans-(3-2, 2 dichlorvinyl)-2,2 dimethylcyclopropane-1-carboxylic acid (cis-trans-DCCA), 4-fluoro-3-phenoxybenzolic acid (F-PBA), and 3-(2,2-Dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, which are excreted in urine. The carbaryl metabolite 1-NAP showed higher levels in the older population than in the younger ones.[97] In two observational studies performed in the USA, 3-PBA was detected in more than 60% of children's urine samples.[99] Studies found high frequencies of pyrethroids in the tested USA population, and the levels were higher than those found in Germany.[101]

METALS

Arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are among the metals suspected of possessing carcinogenic and endocrine-disrupting effects and are known to cause neurobehavioral and reproductive disorders.[102,103] Large quantities of metals are released during mining and smelting processes. Volcanic eruptions, forest fires, weathering of rocks, burning of fossil fuels, waste incineration, soil erosion due to deforestation, agriculture, manufacture of mercury-containing medical equipment and electronics are some of the sources of metal released into the environment. Seafood is known to accumulate elevated metal levels. The inorganic forms of As are highly toxic, and the trivalent form is the most toxic. High concentrations of As in drinking water have been reported in Bangladesh, India, and China for some time, and many new reports are observed from Afghanistan, Pakistan, Taiwan region, Argentina, Mexico, and many other countries.[104–107] Most of the population is ubiquitously exposed to As at levels which may not cause adverse effects.[108] Smoking is considered an important pathway of Cd exposure due to uptake of Cd by the tobacco plantation. Inorganic mercury released into the environment is methylated, which bioaccumulates in the tissues of organisms. Nearly, 70% of blood Hg is found in the organic form due to consumption of contaminated fish, especially carnivorous fish.[109] Methylmercury (MeHg) can form thiol complexes with the cysteine, which increases its mobility across cell membranes.[110] Hg in the form of vapour is highly diffusible and can readily pass the blood-brain and placental barriers.[110] Leaded gasoline and paints have contributed largely toward lead exposure. Mixtures of toxic metals are reported to project supraadditive effects.[102] Toxicity studies of As, Cd, and Pb coexposure showed modest to severe adverse effects compared with single element exposure.[111]

Exposure is monitored by measuring levels of metals in urine, blood, hair, and nails.[106] A major route of As excretion from the human body is through urine, accounting for nearly 60%-70% of the dose. The As blood level is considered a nonspecific biomarker, as it has a very short half-life.[108] Up to 40%-60% of inhaled Cd reaches the circulatory system via the lungs, whereas 5%–10% of ingested Cd is absorbed, which may lead to toxicity due to continual accumulation in a long-term exposure.[112] Blood Cd levels mainly reflect current exposure, and urinary Cd levels reflect the body burden.[113] A good correlation between blood-Cd and urine-Cd has been demonstrated, and either biomarker can be used for Cd estimations in biomonitoring.[114] The concentration of total Hg in blood is measured to study the general population exposure, whereas inorganic Hg is measured in urine to study occupational exposure. Lead concentration in whole blood is the primary biomarker of lead exposure. Techniques such as atomic absorption spectrometry, atomic fluorescence spectroscopy, and atomic emission spectrometry have been conventionally applied as individual techniques due to their simplicity and cost-effectiveness. Similarly, these techniques have been coupled to GC, LC, and capillary electrophoresis for enhanced element separation.[115] Owing to the demand of extremely low detection limits, high specificity and sensitivity, inductively coupled plasma mass spectrometry has lately been widely applied in biological samples for single- or multielement analysis.[115,116] Urine and blood samples are acid-treated, usually with nitric acid, incubated and diluted with deionised water prior to analysis. Nail and hair samples are usually washed with appropriate solutions, digested with nitric acid, perchloric acid, or tetramethylammonium hydroxide followed by incubation for several hours.

Elevated urinary As levels have been reported from India, Bangladesh, and Taiwan region due to contaminated drinking water.[104,105] Background urinary As levels and its metabolites in the USA and Europe are substantially lower (10 $\mu g\,l^{-1}$) compared with the Asian continent.[105,117] The CDC fourth national report stated a mean total urinary As of 8.2 $\mu g\,g^{-1}$ creatinine in the USA population.[118] Analysis of nails of the general population revealed high As levels in the regions affected by contaminated drinking water in Mexico and Taiwan region.[119] Due to traditional food consumption, elevated As levels were reported in the Canadian Inuits.[120] A study in China reported high urinary As levels in the population of metal-exposed regions due to contaminated drinking water and indoor burning of metal-contaminated coal.[121,122] Studies have suggested that 50% of As intake of the South American population is due to contaminated food sources.[123] An alarmingly high exposure to As has been reported in the monitored children and maternal-foetal cohort in South America.[123] The background Cd levels among nonexposed general population in blood is less than 0.5 $\mu g\,l^{-1}$; in urine, it is less than 0.5 $\mu g\,g^{-1}$ creatinine.[112] Cd levels increase with age.[113] Swedish studies have reported women to have higher Cd levels due to general iron depletion.[113] Studies have showed that a group vulnerable to Cd exposure is the population having a high vegetarian diet or a high intake of shellfish.[114] In China, compared with a control group, higher urinary Cd levels were reported in people consuming rice from contaminated fields and domestic usage of metal-containing coal.[121] In the USA, adult women showed higher blood levels of Hg than young children; among children, girls had higher levels than boys.[96] It is evident that fish and shellfish consumption causes elevated Hg levels.[124–126] Studies have shown that the population of high fish-consuming regions

of Canada and the USA had higher Hg levels than other countries, and South American countries are at greater risk of Hg exposure due to traditional fish consumption and gold-mining activities.[124] Arctic studies report decreasing Hg levels in Canadian Inuits compared with older studies.[127,128] Inuit mothers of Arctic Canada had a blood Hg level three times higher than non-Aboriginal mothers.[128] Active involvement in gold-mining activities, contaminated fish consumption, and use of contaminated cosmetic products has led to an elevated body burden of Hg in Africa.[92] Numerous reviews have discussed Hg exposure due to gold mining in the Amazonian region, mainly in Brazil.[109,126] The total Hg levels in the hair of the Brazilian Amazonian population are higher than other South American populations.[129] Since the rapid industrial growth in Asia, increasing Hg levels are being reported.[130,131] A substantial decrease in blood lead levels (BLLs) is evident in the Western countries in the past 2 decades due to the use of unleaded gasoline and lead-free paints. Studies have shown decreased Pb levels in the USA population in the past 30 years.[96,132] BLL increased gradually with age and in immigrant USA populations living in urban areas; children especially showed higher BLL.[96,133] Several studies have shown that the Mexican population bears a higher risk of lead exposure due to traditional practices, housing in urban settings, and usage of leaded paints and lead-laced ceramics and pots.[133,134] Similar trends were observed in HBM studies in Canada.[70] Compared with other parts of the world, Brazil reported high BLL, with a major contribution to exposure coming from battery and recycling plants.[135] Moderately high BLL were observed in Canadian and Greenland Inuit mothers, and lead exposure was mainly from shots used in hunting for traditional food.[127] The current exposure limit of 100 $\mu g\,l^{-1}$ BLL is considered excessive for vulnerable groups, such as infants and children, due to their higher gastrointestinal absorption and less effective excretion.[136] The average BLLs of <20 $\mu g\,l^{-1}$ in Japanese children are one of the lowest reported in the world.[137] Recent reports from China suggest decreasing trends of BLL and prevalence of lead poisoning in children; however, the levels are still high compared with developed Western countries.[138] Significant corelations between BLL and breast milk levels are evident; however, the reported low lead levels in milk appear to pose the least risk to infants.[139] Mean BLL in battery manufacturing and recycling plant workers in developing countries was 470 $\mu g\,l^{-1}$ and 640 $\mu g\,l^{-1}$, respectively, which is substantially higher than similar profession workers in the USA and UK.[140]

POLYFLUORINATED COMPOUNDS

PFCs have exceptional physicochemical properties to make the surfaces of substances water and oil resistant. PFCs are extensively used in the surface coatings of cooking pans, food containers, electronic devices, cosmetics, surfactants, and fire-fighting foams. The PFCs of concern are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) because they are persistent and found in humans and the environment. PFOS, its salts and perfluorooctane sulfonyl fluoride (PFOS-F) have been added to the Stockholm Convention list of the new persistent organic pollutants since 2009.[141] PFOS and PFOA have been replaced by other PFCs such as perfluorononanic acid, perfluorodecanic acid, and perfluoroundecanoic acid, and these new compounds start to show increasing levels in humans.[142] Other perfluoroalkyl acids reported in humans are perfluorooctane sulfonamide (PFOSA), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), 2-(N-ethylperfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH or PFOSAA), perfluorododecanoic acid, perfluoropentanoic acid, perfluorohexanoic acid, and perfluorobutane sulfonate (PFBS).[143]

Human exposure to PFCs can occur via contaminated indoor and ambient air, drinking water, and food.[144] PFCs enter food via food packaging, food preparation in PFC-containing cooking utensils, and environmental contamination of food-producing animals and plants.[145] The tolerable daily intake set by European Food Safety Authority is 150 ng kg^{-1} b.w. per day for PFOS and 1500 ng kg^{-1} b.w. per day for PFOA.[146] Internal exposure of PFCs in humans has been measured in blood serum[142,147] amniotic fluid[57] plasma, liver, cord blood, breast milk, and seminal plasma.[148] PFCs are known to form conjugates with proteins in blood; hence, the PFC presence in maternal breast milk is limited.[144] LC-MS/MS has become a standard method for PFC analysis adopted by most laboratories.[149] Sample preparation methods follow treatment of serum samples with enzyme and organic solvents for the removal of proteins and supernatant injected in LC-MS/MS system.[142] A similar method was also applied for PFC analysis in amniotic fluid samples.[57] One method used protein precipitation of samples followed by UPLC-MS/MS technique for the high throughput analysis of cord blood samples.[150] Sample extraction with ion-pairing liquid extraction, liquid extraction without ion pairing, SPE, or direct injection of samples has also been described.[151]

Higher PFOS levels have been reported in Canada, the United States, and Europe compared with Asia and the Southern Hemisphere.[144,151] Monitoring of PFOA in the USA population revealed that, except for the occupationally exposed fraction, the PFOA levels above background were found only in the population from Minnesota and West Virginia/Ohio, relating it to a contaminated water supply.[152] Perfluorohexane sulfonic acid (PFHxS), perfluorononanic acid, PFOA, and PFOS were detected ubiquitously in the USA population.[14] In addition, PFCs measured in the general population from Japan, Denmark, and Canada demonstrated widespread PFC presence.[153] Many studies have found no indication of age-related exposure; however, a few studies in Germany, the United States and Australia observed PFOS and PFOA to be higher in the older population.[144] Dietary exposure to PFOS and PFOA has been suggested as a significant exposure pathway.[144] PFC levels reported in Greenland Inuits were among the highest due to traditional fatty foods.[142] The newly introduced PFC levels in the Inuits from the same study were comparable to Europe and the USA determination of PFCs in pregnant women's cord blood and amniotic fluid has been performed to assess foetal exposure.[57,148,150] Since the voluntary termination of PFOS production by many developed countries, declining trends have been observed generally;[142] however, on the contrary, a marked increase of PFOS levels is observable in developing countries. Time-trend studies from the past 2 decades exhibited variation, with an increasing trend in Norway, a limited increase in Denmark, and a decreasing trend in Germany.[57] A considerable increase in PFOS and PFOA levels was observed in the past 15 years in China.[144,154]

PARABENS

Parabens are a class of suspected weak endocrine-disruptor chemicals possessing antimicrobial properties. Parabens are used widely in pharmaceuticals, cosmetics, and personal care products. Common parabens are comprised of methyl, ethyl, n-propyl, iso-propyl, n-butyl, and iso-butylparaben. Detection of these compounds in breast tumor cells[155] raised concern over the safety of these compounds, suspecting them to be carcinogenic. The EU allows a maximum of 0.4% of a single ester and 0.8% in a mixture of esters in cosmetic products (Council Directive 76/768/EEC on cosmetic products).[27]

Human exposure to parabens is caused via absorption through skin or ingestion or by the use of products containing parabens. There is a limited

understanding of the toxicokinetics of parabens in humans. Parabens are primarily metabolised by humans into *p*-hydroxybenzoic acid and further form glucuronide and sulphate conjugates, which are excreted in urine. Parabens absorbed through skin can be incompletely metabolised, with part of the compound remaining unchanged.[155] *p*-hydroxybenzoic acid is considered a nonspecific biomarker for paraben determination;[156] hence, it is recommended to determine free parabens in addition to all conjugated urinary species as valid biomarkers. Determination of parabens in humans has been reported in urine,[157,158] serum,[159,160] seminal plasma,[160] and breast tumor cells.[155] The most common technique of choice for the determination of parabens is LC-MS/MS.[156,160] The sample is subject to an initial treatment with β-glucuronidase/sulphatase, then incubation and extraction by manual or online SPE technique.[156,160]

There are a limited number of studies available on human exposure to parabens. A study measured urinary parabens in American adults reporting methyl and *n*-propyl parabens in more than 96% of the samples.[157] In the USA population, adolescent and adult women showed higher urinary parabens levels than males, indicating the use of cosmetics as major exposure pathway.[156] It is suggested that different ingredients in cosmetics may form interactive mixtures and increase uptake of parabens as well as other compounds.[161] Increased urinary levels of butylparaben were reported after the topical application of butylparaben containing personal products on male subjects.[158] The determination of parabens in urine, serum, and seminal plasma samples from 60 healthy Danish men showed ubiquitous presence of most of the parabens with significant correlations between all the matrices.[160] The studied Danish population had two and a half-fold lower urinary parabens levels than the USA population.[160] More metabolism information is required to relate the determined biomarkers with exposure.

ULTRAVIOLET FILTERS

Ultraviolet (UV) filters are commonly used in sunscreen products, cosmetics, and personal care products either to provide protection to consumers or to prevent the degradation of the products by UV exposure. UV filters are also used in plastics and other household materials to prevent decolouring. Animal studies suggest that common chemical UV filters such as benzophenones (BP2 and BP3), 4-methylbenzylidene-camphor, and octyl-methoxycinnamate possess endocrine-disrupting potential.[162,163] However, there is limited knowledge regarding their influence on humans, which is debated.[164] Other commonly found chemical UV filters are 2-cyano-3,3-diphenyl acrylic acid, 3-benzylidene camphor, homosalate, 2-ethylhexyl 4-dimethylaminobenzoate (OD-PABA), and 4-aminobenzoic acid (PABA).[165] The European Union Cosmetics directive permits 26 UV filters for commercial use.[27]

UV filters have been measured in human plasma,[166] urine,[166] and breast milk.[167] Occasional determinations of UV filters have also been performed in matrices such as human feces and semen.[168] Analytical technique commonly described for the determination of UV filters in biological samples is HPLC with UV[166] or MS detection.[167,169] The GC-MS technique has also been reported [167] but usually requires extensive sample preparation. The typical sample preparation method for urine samples follows enzymatic hydrolysis by β-glucuronidase at controlled pH and incubation for several hours followed by SPE.[168] Another described method involves the addition of an organic solvent to the incubated sample, centrifugation, and evaporation of the separated organic phase followed by redissolving in MeOH, which is injected into the system.[169] Plasma, serum, or breast milk samples are mixed with organic solvents and centrifuged to precipitate proteins prior to hydrolysis. Detailed reviews of sample preparation and analytical techniques for UV filter analyses have been published recently.[168,170]

BP3 was detected in 96.8% of urine samples in the USA population, which was related to the use of personal care products, explaining the gender and racial/ethnical difference of exposure levels.[171] Whole-body application of UV filters in experimental studies in Denmark and Sweden reported BP3, 4-methylbenzylidene-camphor, and octyl-methoxycinnamate in plasma and urine samples.[165] Studies on pregnant women/mother-child cohort in the USA, France, and Switzerland reported a number of UV filters in urine and breast milk samples, suggesting exposure to fetuses and infants at early developmental stages.[165,172,173] No correlations were found between the UV filters in the milk samples of Swiss mothers and age, body mass index, or eating habits.[167]

ORGANIC SOLVENTS

Organic solvents are volatile organic compounds found in urbanised areas. Commonly used industrial solvents include chlorinated solvents, petroleum hydrocarbons, and oxygenated hydrocarbons. Dichloromethane, chloroform, and perchloroethylene are used for degreasing in industries and commonly used in the dry cleaning of clothes and are suspected to have carcinogenic, teratogenic, and mutagenic effects on humans.[174] Solvents such as ethylene glycol ethers, toluene, xylene, and styrene are used in paint industry, adhesives, resins, and plastics. They are suspected to bind to estrogen receptors, cause menstrual disorders, and interfere with reproductive hormones in humans.[163] Solvents such as toluene are popularly used among nail technicians, which are linked to causing endocrine disruption.[175] Benzene and formaldehyde are classified as human carcinogens by the World Health Organization, and toluene, xylene, and ethylbenzene are mentioned as affecting human health causing respiratory and neurological effects by the USA EPA.[176] Most organic solvents can pass the placental-fetal barrier, which could be hazardous to the developing fetus. Among other occupations, paint industry workers, petrochemical and gas station workers, tannery and footwear workers, chemical factory workers, dry cleaners, and nail-saloon workers can be considered at high risk to organic solvents exposure.

Exposure to cigarette smoke, traffic exhaust, and contaminated air are major sources of exposure to organic solvents other than occupational exposure. Organic solvents have been measured in human alveolar air,[177] blood,[177–179] and urine.[178–181] Analysis of unmodified solvents in urine is regarded as specific biomarkers of exposure and correlate to occupational exposure.[178,182] GC has been commonly applied for determination of organic solvents in biological samples with detectors, such as a flameionization detector[178,181] and MS.[183,184] LC-MS/MS is also efficiently applied for determination of organic solvents after filtration and acidification of urine samples.[180] The majority of sample extraction methods described for GC-MS analysis of blood and urine samples is headspace-solid phase microextraction.[179,182,184] Automatic headspace samplers have also been employed to directly inject headspace gases into GC-MS systems.[183] Different techniques for exhaled air sampling are described in the literature.[185]

Ethylbenzene, methyl-tert-butyl-ether, toluene, and xylene were found ubiquitously in blood samples in the U.S. population.[14] Urinary styrene metabolites mandelic acid and phenylglyoxylic acid were detected widely in a study performed in the Italian population.[180] Benzene, toluene, and xylene were determined in end-exhaled air samples of

101 primary school children exposed to indoor cigarette smoke and vehicle smoke in Turkey.[185] A study performed on drycleaners detected dichloromethane, chloroform, and tetrachloroethene ubiquitously in urine samples, showing an increasing trend of solvents with an increased length of work shift.[174] Toluene was reported in alveolar air, blood, and urine of male workers from a chemical factory.[177] Dermal exposure study in workers of a petrochemical plant detected benzene in all the samples and toluene in 93% of samples.[181] In Italy, a decreasing trend was observed in the levels of organic solvents in the paint industry workers in the past 2 decades due to usage of safety equipment and following of regulations.[184] Occupational exposure studies in paint factory and footwear factory workers reported the presence of toluene, ethylbenzene, and xylene in urine and blood samples showing good correlation between volatile organic compounds (VOCs) present in air, blood, urine and urinary metabolites in samples.[186] The mean urinary levels of toluene biomarker hippuric acid in Mexican tannery workers were three times lower than those found in a Chinese shoe factory.[187]

QUESTIONS FROM THE PANEL

Q1: How to select new exposures for monitoring?

A1: In the HBM of EDCs, the scientific society often concentrates on a limited number of compounds at one time. The methods for monitoring metals such as lead and mercury were developed early. After the discovery in the 1960s of high levels PCB and other persistent organic pollutants in wildlife marine animals,[188] HBM of these compounds was performed for many years. Lately, scientists have focused on other compounds such as PBDEs, PFCs, phthalates, and bisphenol A. However, development of HBM methods for new compounds is rarely presented because of difficulties in finding such new compounds.

The authors of this review work at an occupational and environmental department. Many of the projects performed at the department are initiated by patients with some type of disease coming to the physicians, and a new type of exposure can be identified at the work place. Similarly, in the scientific literature, such new exposures can be identified through case reports.

The easiest way to find new substances would, of course, be if the industry itself would be more open with the introduction of new substances. However, it is often impossible to obtain such data. Thus, it is often a useful to attempt to understand the way the industry thinks. For example, one strategy the industry often applies is a minor modification of current chemicals that have obtained attention for its toxicity. Such examples are the substitution of DEHP with DiNP, which constitutes the introduction of a single CH2-group into the alcohol chains of the DEHP. Another example is introduction of a CF2-group into PFOA to obtain perfluorononanic acid, a compound with a rapid increase during the last years in serum from the general population. It is, therefore, important to develop HBM methods analysing groups of chemicals and not only single compounds.

In Europe, the new Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) legislation was adopted for all of Europe on the first of June 2007. According to REACH, all chemicals produced within or imported to Europe and exceeding one metric ton should be tested by the industry with respect to health and environmental aspects and then registered by the European Chemicals Agency (ECHA). The data regarding these registrations can be obtained at ECHAs home page (echa.europa.eu). This information provides scientists a possibility to find new large-scale chemicals with endocrine disrupting properties. In addition, close contact with other national or international agencies may provide input to discoveries of new chemicals for HBM. In Sweden, there is a group of researchers involved in HBM and several Swedish agencies, including Swedish Environmental Protection Agency, Swedish National Food Agency, Medical Product Agency, Swedish Chemicals Agency, and the National Board of Health and Welfare that meets regularly discussing new HBM needs.

One possible method of finding a new EDC is to study animals at the top of the food chain such as seals and eagles. It was in this way that PCB exposure was discovered.[188] A more sophisticated but also very time-consuming way of identifying new EDCs is through analyses of different matrices from the environment, for example, water from rivers, in which biological activity can be measured, a so-called effect-directed analysis.[189] The compound in the matrix can then be separated in several steps, and the chemicals in fractions containing the activity can be identified by different methods.

Q2: Windows of exposure are extremely important in EDC. It appears that the greatest effects are "*in utero*" or peripubertal and yet may not be fully expressed until adulthood. It would be useful to discuss biomonitoring scenarios where exposures in the key windows would be collected for future study as the individuals attempt to reproduce. As exposome science is emerging, how do we monitor and put into perspective lifelong, ever-changing, exposure milieus?

A2: The first thing to consider when exposure strategies should be developed is the half-lives of the monitored compounds. Among the EDCs, there are several compounds with a very long half-life such as the PCBs and PFCs. The levels of those chemicals only vary a little over long-time periods. Therefore, at constant exposure, a steady state is first reached after three half-lives. Thus, for many PCBs and PFCs, one cannot expect to have reached the steady state even at 20 years of age. Furthermore, in periods where the levels can be expected to change more rapidly, such as during breastfeeding of babies, it is possible to develop models to calculate these changes. This together makes the sampling strategies easier to develop, and only a rather small number of samples are required to obtain a reasonable good estimate of the lifelong exposure milieus.

However, for many EDCs, the half-lives are very fast, with some consisting of just hours, such as those for phthalates, parabens, and bisphenol A. This makes it more difficult to develop HBM methods for an accurate estimate of the lifelong exposure because several samples are needed to obtain a good estimate of the exposure even during a short period of time. Therefore, a careful evaluation of the timing of the sampling during critical windows is very important for these chemicals. The identification of such critical windows must be based on pathophysiological and toxicological information; however, the discussion of how such data can be obtained is beyond the scope of this review. On the contrary, the need to analyse many samples makes it necessary to develop rapid and simple methods for the analyses of these compounds. The use of more rapid work-up procedures and direct injection of urine and protein depleted serum using analyses by LC-MS/MS is such techniques that has been introduced lately.[142]

The use of biobanked samples is important for an accurate estimate of the lifelong exposures. Lately, there has been an acceptance for this on a governmental level and many new biobanks are now created worldwide. This will be of great importance in the future. However, many biobanks have been created by far-sighted individual researchers. Such biobanks can be taken at a very critical window during pregnancy, for example, from rubella screening programs.

However, one problem with the creation of biobanks is that some chemicals are not stable during storage. An alternative to storage of samples can be to analyse the samples almost immediately, for example, through the direct injection of urine of protein-depleted serum and the collection of high-resolution time of flight (TOF) spectra with LC-MS

equipment.[190] These chromatograms can then be stored, and when new samples are later collected, these can be compared to the old ones with a method such as the partial least square analyses, and compounds with an increasing trend can be identified. This might also be a way to identify new chemical that should be monitored, as discussed for **Q1**.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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