

Applicability of non-invasively collected matrices for human biomonitoring

Roel Smolders, Karl-Werner Schramm, Marc Nickmilder, Greet Schoeters

Email: roel.smolders@vito.be;

schramm@helmholtz-muenchen.de;

marc.nickmilder@uclouvain.be;

greet.schoeters@vito.be

Abstract

With its inclusion under Action 3 in the Environment and Health Action Plan 2004-2010 of the European Commission, human biomonitoring is currently receiving an increasing amount of attention from the scientific community as a tool to better quantify human exposure to, and health effects of, environmental stressors. Despite the policy support, however, there are still several issues that restrict the routine application of human biomonitoring data in risk assessment. ~~One of the most main~~ restricting issues ~~being is~~ the need to routinely collect human samples for large-scale surveys. Particularly for small children and babies, the collection of invasive samples suffers from ethical and practical limitations. Unfortunately, they are often most susceptible to the influence of environmental stressors, and invasive sampling should be minimised. This may severely ~~which~~ hamper the implementation of human biomonitoring as an instrument for risk assessment and management.

~~Unfortunately, these are the very populations most susceptible to the influence of environmental stressors, and for whom invasive sampling should be minimised.~~ Children, pregnant women, elderly, or chronically-ill people however are among those that would benefit the most from non-invasive, repeated or routine sampling. ~~Because of these concerns, it can be argued that~~ Therefore, the use of non-invasively collected matrices for human biomonitoring should be promoted as an ethically appropriate, cost-efficient and toxicologically relevant alternative for many biomarkers that are currently determined in invasive matrices. The current paper provides a review of the most applicable non-invasively collected matrices ~~that are~~ currently ~~utilised~~ in human biomonitoring. For several non-invasively collected matrices, an overview of existing biomarkers of exposure and effect is presented, which are compared to biomarkers in other matrices. The main aim of the publication is to provide readers with an insight into the current state-of-the-art on the

use of non-invasively collected matrices for human biomonitoring, and to identify -illustrate how they could be used for further development. ed as matrices in environment and health research.

Background

The recent adoption of human biomonitoring (HBM) as Action 3 in the Environment and Health Action Plan 2004-2010 of the European Commission [1, 2] has motivated the implementation and application of HBM in European environment and health research. Projects within the Sixth Framework Programme of DG Research, such as the ESBIO project (Expert team to Support biomonitoring in Europe [3]) or the INTARESE project (Integrated Assessment of Health Risk of Environmental Stressors in Europe [4]) have further improved the visibility of human biomonitoring as a relevant instrument in environmental al-and health research and integrated risk and health impact assessment, and have stressed the need for a harmonized European approach to HBM. The current paper reflects the work that was done in the context of the INTARESE project, a Sixth Framework Project. INTARESE that brings together a broad array of scientists in the areas of epidemiology, environmental sciences and biosciences to collaborate on the development and application of integrated environmental health impact assessment. This is defined as a methodology for assessing health-related problems deriving from the environment, and health-related impacts of policies that affect the environment, in ways that take into account the complexities, interdependencies and uncertainties of the real world.

Within the context of the INTARESE project, we perceived an overall lack of knowledge on the general HBM was identified as a relevant data source for integrated assessments. However, there was some uncertainty about the methodology and potential application of

HBM data in integrated health impact assessment. Therefore, a literature review was undertaken on past and current developments, potential and applicability of non-invasively collected matrices for HBM, and its relationship with integrated risk or health impact assessment.

While policy support for HBM and its applications in environment and health research is strong, there are still many issues that currently restrict the routine application of HBM data in integrated risk or health impact assessment. One of the most important issues is the obvious need to collect human samples, often invasively. This suffers from ethical and practical constraints, particularly for small children and babies, from whom blood samples typically need to be collected. The European Commission's SCALE Initiative (Science, Children, Awareness, EU Legislation and Continuous Evaluation) has, however, specifically identified children as a main target population, so including this subpopulation in any HBM project is a priority [5]. Likewise, ~~non-invasively~~ sampling non-invasively collected matrices is preferable in ~~susceptible-vulnerable~~ groups as well, such as pregnant women, elderly, or chronically-ill people.

Additionally, repeated or even routine biomonitoring may be desirable for the efficiency evaluation of risk management options and efficacy of environment and health policies. In the case of chromium biomonitoring for occupational exposure for example, an expert panel suggested that a second, or if necessary, third spot (or 24-hour) urine sample was needed before it could be concluded that a person may be routinely overexposed [6]. For short-lived chemicals such as volatile organic compounds or agricultural pesticides, average exposures may not reflect peak exposures arising through infrequent exposure episodes. Repeated sampling of high-exposure subjects provides more insight into the true nature of these episodes and of their toxicological consequences [7]. Increased HBM efforts may also prove useful to identify areas of high exposure and delineate so-called hot-spots. Additional

sampling may provide further information on the specific cause of exposure. ~~I, and~~ increasing the array of biomarkers measured may also further clarify the link between exposure, dose and response [8, 9].

Finally, because non-invasively collected matrices need less specialized personnel for sampling, costs associated with large sampling designs may be significantly reduced. While blood sampling generally requires the contribution of medical doctors and trained nurses, for example, non-invasively collected matrices often do not require this level of training. Even more, Rockett et al [10] investigated the applicability of home-based collection of non-invasive bio-specimens as an alternative sampling option for the United States' National Children's Study. While they concluded that there were a number of caveats that need to be taken into account in the study protocol, home-based collection of bio-specimens is certainly a viable option. Home-based collection additionally, and offers the additional advantage that participant rates would be higher as it would be less troublesome for participants to donate samples.

Because of these advantages, there is a strong case for non-invasively collected matrices for human biomonitoring ~~to be promoted~~ as an ethically appropriate, cost-efficient and toxicologically relevant alternative for many of the biomarkers ~~that are~~ currently determined in invasive matrices. This paper reviews the most applicable non-invasively collected matrices that are currently utilised in human biomonitoring. For all matrices discussed, existing biomarkers of exposure and effect are presented, and their relationship with other biomarkers, both invasive and non-invasive, is discussed. The main aim of the publication is to provide readers with an insight into the current state-of-the-art on the use of non-invasively collected matrices for human biomonitoring and to identify needs for further development.

~~The main aim of the publication is to provide readers with an overview of the available information on the use of non-invasively collected matrices for human biomonitoring, in order to highlight their potential as routine matrices in environment and health research.~~

Urine

Relevance as a non-invasive biomarker

Urine is probably the most frequently used matrix in humans to quantify the degree of environmental or occupational exposure to environmental pollutants, especially for substances with short biological half-lives [11]. The collection and analysis of urine samples carries no associated risk, and large volumes ~~of up to 800ml at once~~ can at once be gathered per individual [12]. Whilst spot or grab (untimed) samples can easily be collected, 24-hr urine voids can be cumbersome to collect, often resulting in improper or incomplete sampling [11]. Therefore, spot collection of samples is most frequently used in biomonitoring programmes, especially for surveys where large numbers of samples need to be gathered. The major disadvantage of using spot samples is the variability in the volume of urine. ~~Also, and the fact that~~ the concentration of many analytes in urine is related to the extent of diuresis. Hence, there is a need to standardize the results of biomarkers measured in spot urine samples. Urinary creatinine concentrations, gravity, and osmolality are methods that are commonly used to account for these variations in urinary analyte concentrations, and to determine whether a spot urine sample is valid for assessing chemical exposure:

- Frequently, urinary analyte concentrations are standardised by expression per gram of creatinine. This method is the standard method according to Bradman and Whyatt [13]. Urinary creatinine levels may vary by age, sex, ethnicity or body mass index and may not be appropriate for pregnant women or children [11]. These

authors also suggested that in order to improve the interpretability of urinary biomarkers, values should be reported both before and after both-normalisation for creatinine-adjusted and unadjusted concentrations should be reported. WHO [14] has developed guidelines to determine whether spot urinary samples are valid, based on their creatinine concentration. ~~with s~~ Samples with creatinine concentrations < 30 mg/dL or > 300 mg/dL ~~being-are either~~ regarded as either too diluted or too concentrated. However, these guidelines have been questioned recently based on detailed assessment of the role of age, gender and ethnicity [11];

- Another commonly used method to standardise biomarker measurements in urine is to take account of the gravity or relative density of urine. This approach standardises ~~:-analyte concentrations-are standardised~~ based on the ratio between the density of the urine under investigation and average urine density [12].

Biomarkers of exposure

Urine has been used as a matrix for a wide variety of both organic and inorganic compounds for both occupational and environmental exposure. The presence of reference materials and the identification of adequate techniques for the determination of organic xenobiotics and their metabolites in urinary samples have been discussed extensively (see [11], [15], [16] and references therein for more information). Urine is widely used in the occupational setting but also more recently to study impact of environmental contamination. Metals and pesticides with short half lives in the body and compounds that are rapidly excreted have been frequently measured in urinary samples of different age groups of the general population. However due to their short half life, the presence of these compounds or their metabolites in urine often only reflects recent exposure. To obtain information on day-to-day variability in exposure, repeated sampling is needed. Table 1 provides an overview of

some biomarkers of exposure measurable in urine. Due to the specific characteristics of sampling, urine is particularly well-suited for repeated sampling of study participants, on a weekly or daily basis, and even at different times during the day. This advocates the use of urine as matrix for substances with relatively short half-lives, for which multiple samples need to be taken in a short period to adequately describe kinetics. Recently, monitoring of phthalate diesters and their metabolites using urine as a non-invasive matrix has received a lot of attention. These compounds are emerging as ubiquitous compounds that are found throughout the general population [17]. ~~Recent human biomonitoring data have shown that the tolerable intake of children is exceeded to a considerable degree [18], and increasing the number of repeated urine samples was found to be essential to adequately describe exposure and reduce misclassification [19]~~

Nevertheless, ~~use-interpretation of urinary biomarkers e-as-a-biomonitoring-matrix-is sometimes complicated by the circumstance that urinary biomarkers as they~~ primarily focus on metabolites of substances. ~~Often, -and-it is generally-not possible to distinguish between the uptake of parent compounds and environmental metabolites. For example, chlorpyrifos is broken down in the body into a number of metabolites which can be detected in urine. However, these same metabolites also occur as natural products of environmental chlorpyrifos degradation. Therefore, so-it is not possible to distinguish exposure to chlorpyrifos from exposure to its environmental degradates [20].~~

Biomarkers of effect

Urine has a longstanding and well-documented history as a matrix in routine clinical health assessment, where basic urinary analysis includes specific gravity, colour, transparency, pH, protein, glucose, ketones and bile pigments [15]. Alpha(1)- and beta(2)-microglobulin excretion and retinol-binding proteins in urine have frequently been described as ~~a-sensitive biomarkers~~ of renal disfunctioning due to environmental exposure to e.g. cadmium and

other contaminants [21]. Additionally, urine has been used as a non-invasive matrix for the presence of base DNA adducts as biomarkers for carcinogenesis—~~for example~~ in smokers [22]. ~~—while u~~Urinary 8-hydroxy-deoxyguanosine (8-OHdG) has been used as a biomarker of the DNA repair response to oxidative stress and DNA damaging compounds [23].

Recently, there has been increased research interest in the metabolic profiling of biological fluids, for which urine can easily be used. This relatively novel technique, which is generally referred to as metabonomics or metabolomics, encompasses the systematic profiling of metabolite levels and their systematic and temporal changes through effects from diet, lifestyle, environment and genetics, using analytical techniques that are based on NMR spectroscopy and mass spectrometry [24]. Although metabonomics currently is not yet sufficiently developed to be used in large-scale biomonitoring studies, it remains a promising tool in the future for the non-invasive screening of ~~reactive~~ metabolites that are involved in many toxic processes [25].

Correlation with other (non-invasive) biomarkers

While urinary biomarkers of effect are well-documented in literature, most attention on the use of urine as a matrix for biomonitoring still is directed to biomarkers of exposure. Typical concentrations of some ~~metall~~lic elementss and organic compounds in urine are presented in Tables 2 and 3. Compared to other bodily fluids such as cord blood and exhaled breath condensate, urine shows relatively higher levels of cadmium and cotinine. ~~—while~~For ~~concentrations for~~ arsenic and total mercury however, concentrations are lower than those generally observed in cord blood.

Several research projects have found excellent correlations between the presence of metabolites in urine and related health effects: ~~f~~For example, there is generally a good the correlation between hippuric acid (biomarker for toluene) or 1-OH pyrene (biomarker for

exposure to PAHs) and DNA damage [26, 27]. For phthalate metabolites, urinary concentrations have been found to be more informative than blood, serum or milk concentrations in the Swedish population. Urinary concentrations showed lower day-to-day variability and were detected at much higher concentrations than in other matrices [17]. However, others [18] showed that caution is needed when using urine samples from pregnant women to screen for phthalate metabolites. Particularly metabolism and excretion of phthalates varies significantly with changing exposure and/or physiological status during pregnancy.

As mentioned earlier, urinary biomarkers generally reflect metabolites of compounds rather than the pure compound itself. This makes it more difficult to correlate urinary biomarkers with biomarkers of exposure in other matrices. For non-metabolised compounds such as cadmium, correlations between levels in urine and other matrices such as blood appear to be good [28]. The same reasoning holds for correlations between urinary biomarkers and external exposure concentrations. Wang et al [29] have reported good correlations between unmetabolised volatile organic compounds in urine and concentrations in indoor environments, but correlating metabolite concentrations in urine to pure compounds in environmental compartments remains more challenging [30]. If positive correlations are found among different matrices, these are generally improved if urinary biomarkers are ~~corrected for~~ expressed as a function of creatinine level or density.

Validation status

Urine as a matrix has widespread use, for both biomarkers of exposure and biomarkers of effect. Urinary biomarkers have been fully validated for a wide variety of substances, reference materials and internationally recognised standard methodologies are often available, and sampling procedures are well-documented.

Cord blood and placenta

Relevance as a non-invasive biomarker

The use of cord blood or placenta as a non-invasive matrix in biomonitoring is one of the most relevant non-invasively collected matrices with regard to the protection of infants and children from the early adverse effect of environmental contaminants. Although the female uterus is a very safe and protective place for unborn children, the fetus can prenatally come in contact with potentially hazardous substances through the placenta. The placenta can however also act as a barrier by concentrating specific toxicants and thereby to some extent reduce exposure of the foetus [31, 32, 33]. ~~Since~~ ~~it~~ has been widely illustrated that developing organisms, human as well as others, are particularly sensitive towards toxicological insults. ~~Therefore~~, the unborn and newborn child deserves specific protection and attention [34, 35]. This has also been addressed by policy makers, who have identified the need to better protect newborns and children from the negative effects from environmental pollution in for example the PINCHE (Policy Interpretation Network on Children's Health and Environment) or SCALE initiatives. Using placenta and/or cord blood as a matrix for biomonitoring can at the same time provide a picture of the substance exposure history of the mother and the early exposure of the newborn infant.

One problem associated with the use of the placenta as a non-invasive matrix for biomonitoring is the acquisition of a homogeneous tissue sample. Placenta is a complex mixture of blood vessels, chorionic villi and membranes, and metals for example are not uniformly distributed in the different compartments. This raises the question of representativeness of the samples material. Iyengar and Rapp [33] provide an overview of different methodologies to gather placenta samples, but argue that no harmonised approach is practised. The main disadvantages of using cord blood or placenta as a matrix for biomonitoring is ~~obviously~~ that (1) only women are sampled, (2) ~~and that the~~ timing of

births is largely unpredictable and (3) .-Additionally, the collection of cord blood during delivery is a secondary process during delivery. Therefore, and it will not always be practicable to collect standardised, uncontaminated and representative samples.

Biomarkers of exposure

Iyengar and Rapp [36] describe how the placenta can be used to detect the presence of toxic trace elements such as arsenic, ~~cadmium,~~ mercury and or lead. Table 2 provides an overview of the typical range of a number of these trace elements. These authors [36] additionally provide data indicating that concentrations of trace elements can vary 5- to 10-fold due to specific exposure situations, such as living in the vicinity of coal and metal mining and smelting [31].

The placenta may also be a useful matrix to investigate exposure to organic compounds, such as chlorinated hydrocarbons, PAHs, dioxins and pesticides [37, 38]. Table 3 gives an overview of typical concentrations of some organic compounds in cord blood and placenta. Shen et al [35] documented the accumulation of a wide variety of organic compounds including DDT, hexachlorobenzene, or dieldrin in the placenta. Wang et al [36] detected average PCDD/PCDF and PCB levels in placental tissue of 12.8 pg WHO-TEQ/g lipid (95% CI 11.5-14.1 pg/g lipid).

Cord blood has been extensively used to monitor substance exposure of newborns, in combination with the mother's exposure profile. Metals and organic compounds such as pesticides and PCBs have all been extensively measured in cord blood samples. Basically, all biomarkers of exposure, effect or susceptibility that have been developed and standardised for venous blood samples can also be applied to cord blood samples. However, ;-taking into account the specific nature of gathering cord blood and the ~~transfer~~ pharmacokinetics of compounds through the placental barrier need to be taken into account.

Cord blood was identified as the primary matrix to measure exposure to methylmercury,

with also the dried umbilical cord tissue correlating well with cord blood and hair concentrations [40].

Biomarkers of effect

Because of its particular nature, measuring potentially harmful effects of substances in the placenta is of the utmost importance for the protection of the newborn child. In both placental tissue and cord blood, biomarkers for DNA damage have been measured using different methods, including ³²P-postlabeling and ELISA methods [41, 42]. CYP1A1 induction and placental glutathione status have also been proposed as biomarkers of environmental chemical stress [35]. It is alarming that observations have shown that although the estimated PAH dose to the foetus may be 10-fold lower than in the mothers, the PAH adduct levels in the newborns are similar ~~to,~~ or higher than ~~;~~ those in their mothers. This may imply that the foetus may be ~~10-times~~far more susceptible to DNA damage than its mother. ~~This clearly highlights:-hence~~ the urgent need to protect the unborn child and to closely monitor pollutant levels in placenta and cord blood [40].

Wang et al [41] demonstrated in utero effects of placental dioxins and PCBs on thyroid function. ~~These authors found,-with~~ significantly increased T₃, T₄ and thyroid-binding globulin (TBG) concentrations in cord blood correlating to upper-median exposure groups for female but not male infants. Other studies ~~on-the-other-hand~~however found an inverse relation between thyroid hormone concentrations and levels of organochlorine compounds in cord blood. Maervoet et al [42] concluded that, while there are still many gaps in the understanding of the relationship between environmental contaminants in cord blood and the functioning of the thyroid system, ~~it is clear that~~ any interference may adversely affect neonatal neurodevelopment ~~early in life~~. Recently, epidemiological evidence has emerged that shows PFOS and PFOA concentrations in cord blood are related to altered

physiological parameters in newborns, such as head circumference, birth weight or size, or ponderal index [43, 44]

The discovery that placental nucleic acids can be used as a marker for prenatal screening, using real-time quantitative PCR, has recently opened the door for the application of rapidly developing technologies such as toxicogenomics and proteomics. This in the future may lead to, and the development of molecular markers for non-invasive prenatal gene expression profiling of the foetus using placenta and cord blood [45].

Correlation with other (non-invasive) biomarkers

For persistent organic contaminants, good correlations are generally found between paired cord blood or placenta samples and other matrices such as maternal blood, human milk or amniotic fluid. Shen et al [35] however illustrated how metabolites of less persistent organic compounds are found in higher concentrations in placenta than in human milk, which highlights the metabolisation potential of the placenta. A range of typical concentrations of selected metals and organic compounds is provided in Tables 2 and 3.

Cotinine levels are generally significantly higher in newborn cord blood plasma than in paired maternal plasma [46]. Van Oostdam et al [47] found excellent correlations between cord blood and maternal blood samples for pesticides and PCBs in the Arctic. For metals, good correlations are usually observed when metal content of cord blood is compared to paired maternal blood concentrations, human milk or other tissues [40, 48].

Whyatt et al [46] found that the levels of DNA damage from PAHs were higher in cord blood white blood cells than in paired maternal white blood cells. This observation may, which may be an illustration of the reduced detoxification capabilities or decreased DNA repair capacity during foetal development.

Validation status

Biomarkers of exposure and effect are generally well validated for cord blood and placenta measurements, at both a methodological and an epidemiological level. Epidemiological evidence on the effects of transplacental exposure of environmental pollutants on birth outcomes has highlighted that for example levels of PAH-DNA adducts in cord blood are associated with lower birth weight or smaller head circumference [34]. Also oestrogen or progesterone metabolism have been associated with environmental tobacco smoke, dioxins and PCBs [38, 39].

Although the measurement of placental nucleic acids has been established [45], Myllynen et al [38] argue that it is currently too soon to estimate fully the potential of these techniques as validation of these biomarkers is still lacking. Optimal and appropriate biomarkers of placental toxicity are still being sought.

Human milk

Relevance as a non-invasive biomarker

Human milk is a sensitive matrix for the assessment of individual or population exposure to pollution. It is obtained non-invasively and contains a high proportion of lipids. In fact, human milk offers a number of exclusive nutritional and protective properties, which makes it a unique food source for newborn development [49]. Currently human milk is considered one of the most acceptable matrixes for monitoring persistent bio-accumulating toxicants (PBTs). In the lactation period, human milk is a more effective decontamination route than placenta due to its more fatty character [50], especially for primigravidae mothers [51]. Human milk is easy to collect, enriched in ~~lipophilic~~ lipophylic compounds and represents the main exposure source for breast feeding infants [52]. Exposures to chemicals around the home and in processed foods are also easily measured in human milk. As with placental materials, the main disadvantage of using human milk as a matrix for biomonitoring is that

only women can be included in a biomonitoring study, and only at certain periods in their lives. The collection of human milk during lactation is a secondary process, and it will not always be practicable to collect standardised, uncontaminated and representative samples. Additionally, not all chemicals in a mother's body are transferred to human milk, so while human milk reflects exposure to the newborn child, it does not necessarily reflect complete exposure of the mother.

Biomarkers of exposure

Many persistent environmental pollutants, such as polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are widespread and can be found in blood, milk and adipose tissue of human (see Tables 2 and 3 for typical ranges). Chemicals that are ~~lipophilic~~lipophilic and resistant to metabolic degradation have a tendency to accumulate ~~with increasing levels of~~in the food chain and to distribute to fatty tissue and human milk within the human body. Thus, human milk might be a useful matrix to investigate exposure of lipophilic organic compound, ~~such as POPs, which are lipophilic and persistent organic pollutant~~. They bioaccumulate via the food web and cause adverse effects to human health and the environment, and transfer from mother to infant through breastfeeding. High levels of POPs were measured in human milk samples from northern Russia [53]. Anderson and Wolff [54] documented increased serum levels of polybrominated biphenyl, PCBs, hexachlorobenzene, mirex, and lindane and DDE with increasing length of breast feeding in children. Human milk thus seems to be a major source of these pollutants during this period. Alarming, the median daily intake of dioxin levels is 77 pg I-TEQ/kg bw for a nursed 5 kg baby, which is about 50 times higher than the average daily PCDD/F intake for an adult [55]. Also, it has been indicated that the mean daily intake of these compounds by babies is 10-38 times higher than the average WHO tolerable daily intake for lifelong exposure [56]. The exposure of the nursed infant to PAHs via milk has been shown to depend on the daily

cigarette consumption of the mother [57]. Not only persistent organic compounds are present in human milk. Several toxic metals and their derivatives (eg. mercury and methyl mercury, lead, cadmium) also occur [58, 59]. El-Nezami et al [60] illustrated the presence of aflatoxins in human milk and its transfer to the infants.

Despite the detection of a variety of potentially hazardous substances in human milk, it still needs to be stressed explicitly that human milk is by far the best food source for an infant, and the established benefits of breastfeeding by far outweigh the risks of exposure to chemicals [49].

Biomarkers of effect

To our knowledge, no biomarkers of effect have been measured in human milk. However, exposure markers in human milk have frequently been associated with health effects in newborns. The persistent environmental pollutants in human milk samples from general populations have led to concerns that these chemicals may have detrimental effects on the health or development of breast-fed children. Several epidemiological studies reported a relationship between frequent dietary consumption of fish by child bearing-aged women and deficits in the neurological development of their children [61, 62]. Another study observed that bio-persistent chemicals in human milk, (e.g. hexachlorobenzene, p,p'-DDE, methylmercury, and PCBs) are associated with mild neurodevelopment deficits as a possible health hazard in infants. Also neurological interference was increased with the increasing level of contaminants in human milk, and the plasma level of thyroid stimulation hormone were increased [63].

Correlation with other biomarkers

The evaluation of persistent organic compounds in human milk has shown good correlations with biomarkers in other matrices such as placenta maternal blood and amniotic fluid. The serum level of several persistent organic compounds in children increases with the length of

breast feeding and point to human milk as the main source of the pollutants during this period. Nair et al [51] reported that human milk may be the main source of chlorinated pesticide contamination to the newborn as levels typically found in maternal serum and cord serum generally are much lower than in human milk.

When concentrations of pollutants are expressed on a lipid basis, good correlations are generally reported between whole blood, human milk and adipose tissue level [52]. Shen et al [35] documented the accumulation of a wide variety of organic compounds such as DDT, hexachlorobenzene and dieldrin in the placenta. Wang et al [39] detected average PCDD/PCDF and PCB levels in placental tissue of 12.8pg WHO_TEQ/g lipid (95% CI 11.5-14.1 pg/g lipid). Some persistent PBT concentrations in placenta, such as p, p'-DDE, b-HCH, HCB, dieldrin, p,p0-DDT or mirex, can be predicted from milk concentrations, or vice versa, because of their linear relationship. For these compounds, both milk and placenta samples are suitable for biomonitoring. However, prenatal exposure to some less persistent PBTs cannot be predicted as the placenta probably metabolizes them. For these compounds, placenta appears to be more suitable for biomonitoring than milk to assess prenatal exposure [39].

Validation status

Human milk as a matrix has widespread use for biomarkers of exposure. It is easily collectable and fully validated for a wide variety of substances and reference materials. Internationally recognised standards for analysis are often available, and sampling procedures are well documented. The WHO coordinates a regular exposure study to using human milk to quantify the global presence and distribution of persistent organic pollutants.

Within the context of this repeated human biomonitoring program, guidelines for developing a national protocol for sampling human milk have been developed [64, 65].

Exhaled breath

Relevance as a non-invasive biomarker

The air that we breathe can be a major exposure route for a wide variety of potentially hazardous substances, mainly depending on their physico-chemical properties such as partitioning coefficients. Hence, it should not be surprising that a large number of substances that are detectable in ambient air can also be present in expired air in concentrations of biological and toxicological relevance. An advantage of using exhaled breath as a matrix for biomonitoring, particularly for volatile organic compounds, is that this matrix does not generally integrate exposure through a variety of routes, as is the case with blood, urine, or hair, but isolates exposure through air as the sole pathway. For other, less volatile compounds, this may be less restrictive due to the potential rapid diffusion of substances at the blood-gas barrier in the lungs. Measuring exposure and effect in exhaled air may be highly suitable for the characterization of dose at the target organ level, in this case the lungs and respiratory system. Because for most collection systems only tidal breathing is needed, the samples can be collected in a broad range of subjects. ~~U~~ Samples can also be collected from very young children, or individuals with airway diseases.

In general, exhaled breath can be subdivided into two clearly distinct fractions, a gaseous fraction and a fluid, condensate fraction. As will be illustrated further, both fractions have applicability in biomonitoring, though their biological consequences may be very different. On the one hand, the gaseous fraction of exhaled breath is more often used to measure biomarkers of exposure, particularly for volatile organic chemicals (VOCs) such as benzene. The fluid fraction, generally referred to as exhaled breath condensate (EBC), can be

obtained by cooling exhaled air. This fraction ~~and~~ is most frequently used to measure biomarkers of effect, including for example protein or metabolite profiles. However, this division is by no means fixed and examples of biomarkers of exposure and effect will be given for both gaseous and fluid fractions.

Biomarkers of exposure

Probably the best known biomarker measured in exhaled breath is ethanol, which is frequently applied in the context of traffic safety control. In this routinely applied technique, ethanol concentrations in exhaled air are quantified and assumed to be correlated with blood levels, hence indicating potential alcohol intoxication in drivers. The gaseous fraction of exhaled breath can likewise be a good biomonitor for a wide variety of environmental substances. For example, 2,5-dimethylfuran is a good biomarker for smoking status of constituents. Also benzene has been proposed as a suitable indicator for active smoking [66], though there are generally too many other sources of benzene for it properly to function as an indicator of passive smoking [67]. Situations in which benzene proves to be a good indicator of exposure include exposure to automobiles, petrochemical products and combustion processes [68].

The monitoring of metals in exhaled breath is less well documented, although Mutti et al [69] show that toxic metals and transition elements are detectable in EBC, and may have potential in the assessment of the target tissue dose for substances with potential pneumotoxic activity, such as Cd, Co or Ni [69, 70].

A disadvantage of biomarkers in the gaseous phase of exhaled air is that the residence time of substances are generally rather short, in the order of minutes or hours. Hence they only represent short term exposure [66].

Biomarkers of effect

Exhaled breath has received much attention as a suitable clinical matrix for the early detection of pulmonary and respiratory diseases. Generally, the methods used for the detection and evaluation of diseases such as asthma and chronic obstructive pulmonary disease (COPD) are clinical in nature, and of limited value in environment and health research. However, a number of biomarkers of effect have been identified that may offer better insight between the presence of hazardous substances and associated health effects. It has extensively been demonstrated that fractional exhaled nitric oxide (FeNO) levels in exhaled air are higher in people suffering from various pulmonary diseases, including asthma and COPD [71, 72]. A significant amount of research shows that FeNO is a very sensitive and specific marker of pro-inflammation and oxidative stress in the lungs. It offers, ~~with~~ good reproducibility, sensitivity, specificity, and a well-documented dose-response association with different respiratory health effects.

Other biomarkers, such as exhaled breath temperature, pH of EBC, and the presence of cytokines (e.g. IL-4, IL-6, IL-8 and TNF- α), 8-isoprostane or hydrogen peroxide may also be biomarkers of lung inflammation and oxidative stress [71, 73]. Furthermore, it was demonstrated that a combination of biomarkers in exhaled breath (e.g. a combination of biomarker measurement in EBC and FeNO in the gaseous fraction) may increase the applicability, sensitivity and specificity of biomarkers in exhaled breath [71].

Most studies use ELISA kits to measure specific cytokines in EBC, but a very promising tool is the use of proteomics, in which a broad range of proteins can be measured simultaneously. In this approach, protein patterns can be studied instead of ~~a~~ single biomarker endpoints. Particularly within, ~~which might be more useful in~~ clinical practice or environmental biomonitoring, this may be very useful. In a relatively new development, the profiling of low-molecular weight endogenous macromolecules and metabolites in both EBC and exhaled air may have useful applications in terms of a rapid screening or early

warning tool [71, 74]. Using biochemical profiles of these compounds through for example metabonomics in EBC or the use of an “electronic nose” for exhaled air may provide sensitive, rapid and easy-to-use screening techniques [71, 74]. The use of metabonomics may offer additional tools for EBC analysis. ~~Not only~~ may it contribute to in the detection and quantification of known metabolites, but also in the prediction and identification of new markers and unknown metabolites. This approach ~~, which~~ may eventually provide more insight into disease mechanisms. Carraro et al [74] have already illustrated how EBC metabonomics can define the airway biochemical phenotype in patients with asthma. These authors ~~and~~ argue that NMR metabonomics may have a multitude of further applications in pneumonology. Di Natale et al [75] were able to identify lung cancer patients by the analysis of the volatile substances in exhaled air using biosensor technology, usually referred to as an “electronic nose”. Others have illustrated how the VOC profile in exhaled breath of patients with lung cancer [76], pneumonia [77] or diabetes mellitus [78] have distinct characteristics that can be identified with an electronic nose biosensor.

Correlation with other (non-invasive) biomarkers

With regards to biomarkers of exposure, the Total Exposure Assessment Methodology Studies have established strong relationships between exhaled breath concentrations of VOCs and associated personal exposure. These studies ~~, indicating~~ that biomarkers in exhaled breath can provide information on previous exposure as well as current body burden [66, 67]. Nieuwenhuijsen et al [79] also illustrated that the concentration of water disinfection by-products correlated well with concentrations in other matrices such as blood serum or urine. The main problem associated with the correlation between alveolar chloroform and plasma chloroforms for example, is the relatively short biological half-life of chloroform in exhaled air. This example shows ~~, emphasising~~ the need to take into account time since last exposure for volatile organic compounds.

Biomarkers of effect in EBC did not correlate well with those measured in broncho-alveolar lavage (an invasive method to sample the lining fluid of the lower respiratory tract), ~~although~~ ~~†~~ This lack of correlation may be a reflection of ~~the~~ differences in sampling strategy, as EBC samples reflect a much larger portion of the respiratory tract [73]. However, biomarkers of effect measured in EBC did correlate well with clinical observations in patients suffering from a range of inflammatory diseases including asthma and COPD, highlighting the health relevance of the biomarker measurements.

Validation status

A major advantage of measuring biomarkers in EBC and exhaled air is that an immediate quantification of relevant pneumotoxic substances is available. This, ~~which~~ provides an accurate estimate of the active dose at the level of the target organ. Methods of collecting and sampling exhaled breath biomarkers include Tedlar bags, canisters, or even portable real-time detectors. These methods are generally well validated and documented, as are the measurement techniques. The circumstance that sampling matrices are very similar to air and water. They, ~~and~~ do not show extensive matrix effect due to the presence of potentially interfering solutes. This in its turn, limits confounding matrix effects and strengthens the ease of use, sensitivity and efficiency of quantification [70]. Both the European Respiratory Society (ERS) and American Thoracic Society (ATS) have developed recommendations for measuring FeNO, and measurements are highly reproducible if careful attention is paid to technique [71, 72]. ERS and ATS also developed recommendations for the collection and measurements in EBC [80]. However, this method still needs further research (reproducibility of the markers, mechanisms of particle formation, dilution factor, etc) before this technique can be routinely used in clinical practice.

Hair

Relevance as a non-invasive biomarker

Hair as a non-invasive biomonitoring matrix has been successfully used to measure both internal and external exposure to a wide variety of organic and inorganic pollutants [81, 82, 83, 84]. With recent analytical developments, it is currently feasible to measure concentrations of metals such as Zn, Cu, Cr, Hg or Pb in individual hair strands (Table 2) [85, 86]. Also analysis of organohalogenated and other organic pollutants in hair samples has become more and more popular in recent years (Table 3). The attractiveness of using hair as a biological non-invasive matrix is in the fact that there generally are no ethical restrictions in collection with respect to age or gender. Even in newborns, hair samples can be collected in a standardised fashion, without needing to correct for confounding factors, as is the case for creatinine in urine samples for example. As hair grows about one centimetre per month, analysis of hair of different length may reflect cumulative exposure over several months.

Potential constraints on the use of hair include the difficulty in differentiating between internal and external sources of contaminant. The contaminant load may be a result of uptake inside the hair or adsorption to the outside of the hair strand. However, this factor can also be a benefit. Schramm et al [87, 88] indicated that, through a successive washing process, fat and sweat as well as exogenous substances is removed from the surface of hair. Thus, the short-term external and long-term internal contamination can be potentially separated during sample preparation.

Biomarkers of exposure

Environmental organic contaminants have been proven to reach hair via two major routes: endogenous (xenobiotics reach the hair matrix through blood) and exogenous (atmospheric deposition) [83; 89]. Schramm et al [87] first reported the occurrence of PCDD/F on hair, and subsequently identified fast adsorption and a slow desorption of relevant compounds on

hair [90]. These authors also reported partition coefficients between ambient air and hair. Thus, gaseous exposure can potentially be predicted from hair concentrations. Findings by Nakao et al [91] and Stupar et al [92] also show that hair can be used as an indicator for detection of atmospheric exposure and in some cases even for the estimation of corresponding air concentrations.

Under the appropriate washing steps, exogenous contamination can be removed, allowing endogenous contamination levels to be successfully analyzed. As mentioned earlier, refined analytical techniques allow for the detection of metals in single hair strands, while a wide variety of organic contaminants can also be detected in hair. Table 2 provides an overview of typical concentrations of contaminants detected in hair. Probably the best-known use of hair as a non-invasive matrix is in the biomonitoring of organic and inorganic mercury, as hair is by far the best integrator of past exposure [93].

Hair samples have also extensively been used in non-invasive ecosurveillance monitoring, for example in the monitoring of contaminant levels in both domestic animals [94] and wild animals such as hedgehogs [95] or bird feathers [96].

Biomarkers of effect

To our knowledge, there are no examples of biomarkers of effect measured in hair samples. Recently however, RNA isolation from hair follicles has opened the possibilities to use hair for gene expression analysis, which may in the future have relevance for developing signatures of toxicant exposure [97].

Correlation with other (non-invasive) biomarkers

Many authors have addressed the correlation between contaminants in hair and other matrices. Nakao et al [98] found moderate to strong correlations between the contamination levels of PCDD/Fs and Co-PCBs in hair and blood, collected from six donors. Recently, Altshul et al [99] collected human hair samples from 10 volunteers and analyzed the

exposure level of organochlorine pesticides (OCPs) and 57 individual PCB congeners. Strong hair-to-blood correlation ($r=0.8$) of p,p'-DDE and moderate correlations between hair and blood levels of PCB congeners 28, 74, 99, 170, 180 and 194 were found. o,p'-DDE levels were negatively correlated between hair and blood. The other PCB congeners and OCPs showed no or only weak correlations between the two matrices.

For inorganic compounds, Mehra and Juneja [100] reported significant correlations between hair and nail levels of Cd and Pb, Stupar et al [92] found significant correlations between Pb in hair and blood, and Ng et al [101] reported significant correlations between hair mercury and mercury in blood, 24h urine or cord blood samples following a meta-analysis of mercury biomonitoring studies.

Validation status

A review by the Agency for Toxic Substances and Disease Registry highlighted some of the shortcomings of hair as a non-invasive biomonitoring matrix [102]. Although the review recognised that hair is a very useful matrix for identifying historical exposure to contaminants and may have predictive value towards health effects, a large amount of uncertainty remains regarding sampling procedures, quality assessment and control issues, and the lack of reference ranges and dose-response outcomes. In a recent review however, Schramm [88] provides a detailed overview of past research on the development of hair analysis for organic contaminants. This includes analytical procedures, successful applications and their limitations along with sampling requirements, sample processing, extraction procedures, clean-up and analysis methods for organic contaminants. Also, a broad overview of potential confounding factors is presented, including factors such as age, gender, smoking behaviour or the effect of hair treatment and colouring.

Schramm [88] concludes that hair analysis provides an ethically acceptable, inexpensive and easily applicable biomonitoring system for human and animal samples. Furthermore, the

special structure and position of hair makes the elaboration of short-term and long-term exposure of individuals possible. However, there are still some restrictions, which limit hair analysis as a valid monitoring tool for risk assessment. Therefore, it encourages further studies in the following fields:

- Establishment of standardized analytical process, including sampling, washing procedure, extraction, analytical methods and quality control/quality assurance.
- Study of the kinetic and mechanisms by which organic contaminants are incorporated into hair and their retention in the hair matrix, and identification of their metabolites in hair
- Establishment of a database of POPs in human hair
- Exploration of possible dose-response relationships
- Better understanding of hair biology (variations of hair composition with age, gender, race, etc)

Other relevant matrices

Relevance as a non-invasive biomarker

Apart from the matrices discussed above, there are a number of other non-invasively collected matrices that offer great potential for routine application in human biomonitoring. Although their use and applicability is much less documented in literature than the ones previously discussed, some examples and potential future applications are given below.

Biomarkers of exposure

Finger/toe nails: metals have frequently been monitored using fingernails or toenail clippings. Mehra and Juneja [100] reported concentrations of cadmium and lead in toenails from both occupationally exposed and environmentally unexposed subjects, and [103] used the concentration of arsenic in fingernails to confirm chronic exposure of a northern Chilean

population to arsenic in drinking water. Recently, [104] determined 14 different metals in toenail clippings from Arab-Americans in the Detroit area, Michigan, illustrating how toenail clippings could provide insight into lifestyle and demographic factors influencing exposure to metals. Obviously, precaution needs to be taken to avoid metal contamination from the clippers.

Meconium/feecesfaeces: meconium is the earliest stool from an infant, and can be used to check for in utero exposure to substances. Meconium is often used to quantify the neonate's exposure to harmful substances such as alcohol [105], environmental tobacco smoke [106], or other illicit drugs [107]. Meconium has also been used to quantify neonatal exposure to environmental contaminants, mainly organochlorine and organophosphate pesticides [108, 109, 110] and metals [111, 112,113] . For later stages of life, feecesfaeces has been found to represent a suitable matrix for current gastrointestinal exposure to e.g. lead [114].

Saliva: Saliva sampling has been applied for a variety of contaminants and applications. Yuan et al [115] used saliva to determine arsenic speciation and found the concentrations to correlate well with drinking water arsenic. Moreover, participants with increased saliva arsenic concentrations also showed increased odds ratios for skin lesions, an important clinical symptom of chronic arsenic exposure and poisoning. Also other metals, such as lead [116], mercury [117] or several other metals [118] have been detected in saliva, and were often correlated with identified exposure sources. The presence of non-persistent organic chemicals in saliva is well documented, often following occupational exposure of pesticide applicators. Methods to determine chlorpyrifos [119], diazinon [120] or atrazine [121] have been described in literature, and were generally very sensitive in reflecting pesticide exposure and recovery after application. Also phthalates and several of its metabolites have been detected in saliva [122], and could used to estimate oral exposure to phthalates through mouthing of soft PVC baby toys [123].

Biomarkers of effect

Nail clippings: We have not found any examples of nail clippings used for effects monitoring.

Meconium/feecesfaeces: No clear examples of the use of meconium or feecesfaeces as a non-invasively collected matrix to determine health effects have been found in literature. However, exposure to metals and pesticides determined in meconium has previously been shown to be reflective of the neonate's health status, reflected in low birth weight or prematurity [112]. While feecesfaeces has repeatedly been used as a non-invasively collected matrix for medical purposes, screening for diseases of the digestive tract such as inflammatory bowel diseases [124] or colorectal cancer [125], their application in environment and health research is not documented.

Saliva: Saliva as a non-invasively collected matrix has been used to determine health endpoints following exposure to environmental toxicants. Particularly the quantification of cholinesterase activity in saliva, a biomarker for potential neurotoxic effects, has been identified as potential useful indicator associated with exposure to organophosphorus and carbamate pesticides [126, 127]. Additionally, saliva specimens have been used in combination with the Ames test or a Chinese hamster V79 lung fibroblast cell line to investigate the genotoxic effects of smoking and alcohol consumption. The study showed that saliva showed a genotoxic and cytotoxic response, and may be predictive of increased risk for squamous cell carcinomas in the upper aerodigestive tract [128, 129].

Associated with the collection of salivary samples, exfoliated buccal cells have been used to monitor genetic damage in humans using the micronucleus test [130, 131]. This test has been used to illustrate the effects of a broad range of environmental and occupational contaminants, including petroleum derivatives such as benzene [132], ozone [133], or arsenic [134].

Correlation with other (non-invasive) biomarkers

Nail clippings: Mehra and Juneja [100] reported significant correlations between cadmium and lead concentrations in hair and nail clippings. In Nigerian auto-mechanics however, fingernail lead content failed to distinguish among exposed and unexposed subjects, while hair and blood lead levels were significantly different among these participants [135].

Meconium/feces/faeces: In many cases, meconium has been shown to be the most sensitive matrix to analyse fetal exposure to environmental contaminants. Ramirez et al [111] illustrated how the prevalence of mercury in meconium was higher than in maternal fluids, making this a very relevant non-invasively collected matrix to be measured to determine the load of fetal Hg. The authors illustrated that for 78 mother-infant pairs in a community with high Hg pollution, 46.1% of all meconium samples showed detectable amounts of Hg, while this was only 31.6%, 16.7% and 6.4% for respectively infant's hair, cord blood and human milk. Hg in meconium correlated well with levels in the mother's blood and cord blood. Also for a variety of pesticides, meconium showed the highest exposure rates compared to cord blood and infant hair [136].

Saliva: When paired blood and saliva samples were taken, pesticide concentrations generally correlated well among both matrices [119, 120, 121]. For metals, correlations are generally weaker. Urinary mercury levels correlated better with blood levels than with saliva levels [117], and also for lead, only weak correlations between saliva and blood levels were observed [116].

Validation status

Nail clippings: Slotnick et al [135] evaluated the effect of demographic characteristics and nutritional measures on the association between drinking water and toenail arsenic concentrations. Apart from toenail iron concentration, no demographic or nutritional parameters affected the biomarker response. In Kenya however, [136] reported that socio-

economic background, health conditions, dietary habits and urban or rural living had a significant effect on lead, cadmium, zinc and iron concentrations in the fingernails of school children. For arsenic, [137] illustrated that different ethnic groups showed significantly different levels of total arsenic in fingernails, suggestive of a different pattern of arsenic metabolism in different ethnic groups.

Meconium/feces/faeces: Because of the sensitivity of meconium for illicit drug screening, structured screening protocols have been developed [140, 141]. Also for environmental exposure to potentially harmful substances, validation studies have been performed and confounding factors have been defined and described [112, 142].

Saliva/buccal swabs: Because concentrations of toxicants in saliva are often very low compared to other matrices [115, 118], exposure monitoring using saliva is most documented for occupational exposure. The use of buccal cells to detect DNA damage using the micronucleus assay has been the subject of the HUMN project (Human Micronucleus Project), which aims at exploring sources of variability in the assay and resolve key technical issues. This will allow for a reliable comparison of data among populations and laboratories, taking into account inter- and intra-individual variability [130].

Conclusions

The use of non-invasively collected matrices can be a valuable alternative to, or addition for, invasive matrices such as peripheral blood sampling for most contaminants discussed. Generally, there is good agreement between invasive and non-invasive biomarker values. However, the applicability of non-invasively collected matrices is sometimes hampered by incomplete knowledge on, for example, toxicokinetics and validated sampling, sample

treatment and analysis procedures. On the other hand, non-invasively collected matrices can offer substantial advantages for practical and routine implementation. Some of these advantages are, such as increased participation rates, repetitive sampling, more efficient inclusion of susceptible and vulnerable populations, and improved cost-efficiency.

However, as was already highlighted in more detail for hair, several issues may need to be addressed before non-invasively collected matrices can be used for routine HBM in environment and health research:

- Establishment of standardized analytical process, including sampling, extraction, analytical methods and quality control/quality assurance.
- Information on the pharmaco- and toxicokinetic mechanisms by which contaminants behave in the different non-invasively collected matrices
- Further exploration of possible dose-response relationships
- Better understanding of inter- and intra-individual variability of non-invasively collected matrices

Once some of these issues are resolved, Particularly within the context of risk and health impact assessment, the use of repeated sampling of non-invasively collected matrices to evaluate the efficiency of risk management options and efficacy of environment and health policies offers great potential.

Abbreviations

HBM: human biomonitoring; ESBIO: Expert team to Support biomonitoring in Europe;

INTARESE: Integrated Assessment of Health Risk of Environmental Stressors in Europe;

SCALE: Science, Children, Awareness, EU Legislation and Continuous Evaluation; 8-OHdG: 8-hydroxy-deoxyguanosine; NMR: Nuclear Magnetic Resonance; PAHs: polycyclic aromatic hydrocarbons; PINCHE: Policy Interpretation Network on Children's Health and Environment; PCDD/PCDF: polychlorinated dibenzodioxins/furans; TEQ: toxic equivalent; ELISA: enzyme-linked immunosorbent assay; PCB: polychlorinated biphenyls; TBG: thyroid-binding globulin; T₃: triiodothyronine; T₄: thyroxine; PFOS: Perfluorooctanesulfonic acid; PFOA: Perfluorooctanoic acid; PCR: polymerase chain reaction; PBTs: persistent bio-accumulating toxicants; POPs: persistent organic pollutant; DDE: Dichlorodiphenyldichloroethylene; HCH: hexachlorocyclohexane; HCB; hexachlorocyclobenzene; VOCs: volatile organic chemicals; EBC: exhaled breath condensate; COPD: chronic obstructive pulmonary disease; IL: interleukine; ERS: European Respiratory Society; ATS: American Thoracic Society; OCPs: organochlorine pesticides; PVC: polyvinylchloride

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors have taken responsibility for reviewing the use and applicability of different matrices, as described in the different chapters of the review. All authors read and approved the final manuscript.

Acknowledgements

This review was funded by INTARESE, a 5-year Integrated Project funded under the EU 6th Framework Programme – priority 6.3 Global change and Ecosystems.

References

1. Commission of the European Communities. *Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee. "The European Environment & Health Action Plan 2004-2010"*. Brussels, 9.6.2004. COM(2004) 416 final. Volume I. 8 p. Brussels, Belgium, 2004.
2. Commission of the European Communities. *Technical annexes to the Communication of the Commission on the European Environment and Health Action Plan 2004-2010. Brussels 9.6.2004*. COM(2004) 416 final. Volume II. 22 p. Brussels, Belgium, 2004.
3. **ESBIO** [<http://www.eu-humanbiomonitoring.org>]
4. **INTARESE** [<http://www.intarese.org>]
5. **The SCALE Initiative**. [<http://www.environmentandhealth.org/twgim/background.php>]
6. Anderson RA, Colton T, Doull J, Marks JG, Smith RG, Bruce GM, Finley BL, Paustenbach DJ. **Designing a biological monitoring program to assess community exposure to chromium – Conclusions of an expert panel**. *J Toxicol Environ Health* 1993, **40**:555-583.
7. Arcury TA, Quandt SA, Barr DB, Hoppin JA, McCauley L, Grzywacz JG, Robson MG. **Farmworker exposure to pesticides: Methodological issues for the collection of comparable data**. *Environ Health Perspect* 2006, **114**:923-928.
8. WHO. **Biomarkers in risk assessment: Validity and validation**. In: *Environmental Health Criteria* 222. 2001, International Program on Chemical Safety, World Health Organization, Geneva, Switzerland.

9. Hoffmann K, Becker K, Friedrich C, Helm D, Krause C, Seifert B. **The German Environmental Survey 1990/1992 (GerES II): cadmium in blood, urine and hair of adults and children.** *J Expo Anal Environ Epidemiol* 2000, **10**:126-135.
10. Rockett JC, Buck GM, Lynch CD, Perreault SD. **The value of home-based collection of biospecimens in reproductive epidemiology.** *Environ Health Perspect* 2004, **112**:94-104.
11. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. **Urinary creatinine concentrations in the U.S. population: Implications for urinary biological monitoring measurements.** *Environ Health Perspect* 2005, **113**:192-120.
12. Polkowska Z, Kozłowska K, Namiesnik J, Przyjazny A. **Biological fluids as a source of information on the exposure of man to environmental chemical agents.** *Crit Rev Anal Chem* 2004, **35**:105-119.
13. Bradman A, Whyatt RM. **Characterizing exposures to nonpersistent pesticides during pregnancy and early childhood in the National Children's Study: A review of monitoring and measurement methodologies.** *Environ Health Perspect* 2005, **113**:1092-1099.
14. WHO. *Biological monitoring of chemical exposure in the workplace. Volume 1.* Geneva, 1996.
15. Kozłowska K, Polkowska Z, Przyjazny A, Namiesnik J. **Analytical procedures used in examining human urine samples.** *Pol J Environ Studies* 2003, **12**:503-521.
16. Jakubowski M, Trzcinka-Ochocka M. **Biological monitoring of exposure: Trends and key developments.** *J Occup Health* 2005, **47**:22-48.

17. Högberg J, Hanberg A, Berglund M, Skerfving S, Remberger M, Calafat AM, Filipsson AF, Jansson B, Johansson N, Appelgren M, Hakansson H. **Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations.** *Environ Health Persp* 2008, **116**:334-339.
18. Heudorf U, Mersch-Sundermann V, Angerer E. **Phthalates: Toxicology and exposure.** *Int J Hyg Envir Heal* 2007, **210**:623-634.
19. Adibi JJ, Whyatt RM, Williams PL, Calafat AM, Camann D, Herrick R, Nelson H, bhat HK, Perera FP, Silva MJ, Hauser R. **Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples.** *Environ Health Persp* 2008, **116**:467-473.
20. Needham LL. **Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes.** *Environ Health Perspect* 2005, **113**:494-498.
21. Bernard A. **Renal dysfunction induced by cadmium: biomarkers of critical effects.** *BioMetals* 2004, **17**:519-523.
22. Chen HJC, Kao CF. **Effect of gender and cigarette smoking on urinary excretion of etheno DNA adducts in humans measured by isotope dilution gas chromatography/mass spectrometry.** *Toxicol Lett* 2007, **169**:72-81.
23. Staessen JA, Nawrot T, Den Hond E, Thijs L, Fagard R, Hoppenbrouwers K, Koppen G, Nelen V, Schoeters G, Vanderschueren D, Van Hecke E, Verschaeve L, Vlietinck R, Roels HA. **Renal function, cytogenetic measurements, and sexual development in adolescents in relation to environmental pollutants: a feasibility study of biomarkers.** *Lancet* 2001, **357**:1660-1669.

24. Lindon JC, Holmes E, Nicholson JK. **Metabonomics techniques and applications to pharmaceutical research & development.** *Pharm Res* 2006, **23**:1075-1088.
25. Wagner S, Scholz K, Sieber M, Kellert M, Voelkel W. **Tools in metabonomics: An integrated validation approach for LC-MS metabolic profiling of mercapturic acids in human urine.** *Anal Chem* 2007, **79**:2918-2926.
26. Heuser VD, Erdtmann B, Kvitko K, Rohr P, da Silva J. **Evaluation of genetic damage in Brazilian footwear-workers: Biomarkers of exposure, effect, and susceptibility.** *Toxicology* 2007, **232**:235-247.
27. Mielzynska D, Siwinska E, Kapka L, Szyfter K, Knudsen LE, Merlo DF. **The influence of environmental exposure to complex mixtures including PAHs and lead on genotoxic effects in children living in Upper Silesia, Poland.** *Mutagenesis* 2006, **21**:295-304.
28. Shimbo S, Zhang ZW, Moon CS, Watanabe T, Nakatsuka H, Matsuda-Inoguchi N, Higashikawa K, Ikeda M. **Correlation between urine and blood concentrations, and dietary intake of cadmium and lead among women in the general population of Japan.** *Int Arch Occup Environ Health* 2000, **73**:163-170.
29. Wang BL, Takigawa T, Takeuchi A, Yamasaki Y, Kataoka H, Wang DH, Ogino K. **Unmetabolised VOCs in urine as biomarkers of low level exposure in indoor environments.** *J Occup Health* 2007, **49**:104-110.
30. Liljelind I, Rappaport S, Eriksson K, Andersson J, Berghahl IA, Sunesson AL, Jarvholm B. **Exposure assessment of monoterpenes and styrene: a comparison of air sampling and biomonitoring.** *Occup Environ Med* 2003, **60**:599-603.

31. **Baranowska I. Lead and cadmium in human placentas and maternal and neonatal blood (in a heavily polluted area) measured by graphite-furnace atomic-absorption spectrometry.** *Occup Environ Med* 1995, **52**:229-232.
32. **Goyer RA. Transplacental transport of lead.** *Environ Health Perspect* 1990, **89**:101-105.
33. **Iyengar GV, Rapp A. Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 1: Physiology, function and sampling of placenta for elemental characterisation.** *Sci Total Environ* 2001, **280**:195-206.
34. **Perera FP, Rauh V, Trai W-Y, Kinney P, Camann D, Barr D, Bernert T, Garfinkel R, Tu Y-H, Diaz D, Dietrich J, Whyatt RM. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population.** *Environ Health Perspect* 2003, **111**:201-205.
35. **Shen H, Main KM, Virtanen HE, Damggard IN, Haavisto A-M, Kavela M, Boisen KA, Schmidt IM, Chellakooty M, Skakkebaek NE, Toppari J, Schramm K-W. From mother to child: Investigation of prenatal and postnatal exposure to persistent bioaccumulating toxicants using breast milk and placenta biomonitoring.** *Chemosphere* 2007, **67**:256-262.
36. **Iyengar GV, Rapp A. Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 3: Toxic trace elements in placenta and placenta as a biomarker for these elements.** *Sci Total Environ* 2001, **280**:221-238.
37. **Gladen BC, Zadorozhnaja TD, Chislovska N, Hryhorczuk DO, Kennicutt MC, Little RE. Polycyclic aromatic hydrocarbons in placenta.** *Hum Exp Toxicol* 2000, **19**:597-603.

38. Myllynen P, Pasanen M, Pelkonen O. **Human placenta: A human organ for developmental toxicology research and biomonitoring.** *Placenta* 2005, **26**:361-371.
39. Wang SL, Chang YC, Chao HR, Li CM, Li LA, Lin LY, Papke O. **Body burdens of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls and their relations to estrogen metabolism in pregnant women.** *Environ Health Perspect* 2006, **114**:740-745.
40. Grandjean P, Budtz-Jorgensen E, Jorgensen PJ, Weihe P. **Umbilical cord mercury concentration as biomarker of prenatal exposure to methylmercury.** *Environ Health Perspect* 2005, **113**:905-908.
41. Wang SL, Su PH, Jong SB, Guo YL, Chou WL, Papke O. **In utero exposure to dioxins and polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns.** *Environ Health Perspect* 2005, **113**:1645-1650.
42. Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, Nelen V, Baeyens W, Schepens P, Viaene MK. **Association of thyroid hormone concentrations with levels of Organochlorine compounds in cord blood of neonates.** *Environ Health Perspect* 2007, **115**:1780-1786.
43. Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, Goldman LR. **Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth.** *Environ Health Perspect* 2007, **115**: 1670-1676
44. Fei C, McLaughlin JK, Tarone RE, Olsen J. **Perfluorinated chemicals and fetal growth: A study within the Danish National Birth Cohort.** *Environ Health Perspect* 2007, **115**: 1677-1682.

45. Ng EKO, Tsui NBY, Lau TK, Leung TN, Chiu RWK, Panesar NS, Lit LCW, Chan KW, Lo YMD. **mRNA of placental origin is readily detectable in maternal plasma.** *Proc Natl Acad Sci USA* 2003, **100**:4748-4753.
46. Whyatt RM, Jedrychowski W, Hemminki K, Santella RM, Trai W-Y, Yang K, Perera FP. **Biomarkers of polycyclic aromatic hydrocarbon-DNA damage and cigarette smoke exposures in paired maternal and newborn blood samples as a measure of differential susceptibility.** *Cancer Epidemiol Biomarkers Prevent* 2001, **10**:581-588.
47. Van Oostdam J, Donaldson SG, Feeley M, Arnold D, Ayotte P, Bondy G, Chan L, Dewailly E, Furgal CM, Kuhnlein H, Loring E, Muckle G, Myles E, Receveur O, Tracy B, Gill U, Kalhok S. **Human health implications of environmental contaminants in Arctic Canada: A review.** *Sci Total Environ* 2005, **351**:165-246.
48. Raghunath R, Tripathi RM, Sastry VN, Krishnamoorthy TM. **Heavy metals in maternal and cord blood.** *Sci Total Environ* 2000, **250**:135-141.
49. Lawrence RA, Lawrence RM. *Breastfeeding: A Guide for the Medical Profession (fifth ed.)*. Mosby Co., St. Louis, MO, 1998.
50. Waliszewski SM, Molski M, Konarski J. **Organochlorine pesticide levels in maternal adipose tissue, maternal blood serum, umbilical blood serum, and milk from inhabitants of Veracruz, Mexico.** *Arch Environ Contam Toxicol* 2001, **40**:432-438.
51. Nair A, Mandapati T, Dureja P, Pillai MKK. **DDT and HCH load in mothers and their infants in Delhi, India.** *Bull Environ Contam Toxicol* 1996, **56**:58-64.
52. Smith D. **Worldwide trends in DDT levels in human breast milk.** *Int J Epidemiol* 1999, **28**:179-188.

53. Polder A, Odland JO, Tkachev A, Foreid S, Savinova TN, Skaare JU. **Geographic variation of chlorinated pesticides, toxaphenes and PCBs in human milk from sub-arctic and arctic locations in Russia.** *Sci Total Environ* 2003, **306**:179–195.
54. Anderson HA, Wolff MS. **Environmental contaminants in human milk.** *J Expo Anal Environ Epidemiol* 2000, **10**:755–760.
55. Päpke O. PCDD/PCDF: **Human background data for Germany, a 10-year experience.** *Environ Health Perspect Suppl* 1998, **106**:723-731.
56. Wang SL, Lin CY, Guo YL, Lin LY, Chou WL, Chang LW. **Infant exposure to polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls (PCDD/Fs, PCBs) – correlation between prenatal and postnatal exposure.** *Chemosphere* 2004, **54**:1459-1473.
57. Zanieri L, Galvan P, Checcini L, Cincinelli A, Lepri L, Donzelli GP, Del Bubba M. **Polycyclic aromatic hydrocarbons (PAHs) in human milk from Italian women: Influence of cigarette smoking and residential area.** *Chemosphere* 2007, **67**:1265-1274.
58. Chien LC, Han BC, Hsu CS, Jiang CB, You HJ, Shieh MJ, Yeh CY. **Analysis of the health risk of exposure to breast milk mercury in infants in Taiwan.** *Chemosphere* 2006, **64**:79-85.
59. Abadin HG, Hibbs BF, Pohl HR. **Breast-feeding exposure of infants to cadmium, lead, and mercury: A public health viewpoint.** *Toxicol Ind Health* 1997, **13**:495–517
60. El-Nezami HS, Nicoletti G, Neal GE, Donohue DC, Ahokas JT. **Aflatoxin M₁ in human breast milk samples from Victoria, Australia and Thailand.** *Food Chem Toxicol* 1995, **33**:173-179.

61. Fein GG, Jacobson JL, Jacobson SW, Schwartz PM, Dowler JK. **Prenatal exposure to polychlorinated biphenyls: Effects on birth size and gestational age.** *J Pediatr* 1984, **105**:315-320.
62. Stewart P, Reihman J, Lonky E, Darvill T, Pagano J. **Prenatal PCB exposure and neonatal behavioral assessment scale (NBAS) performance.** *Neurotoxicol Teratol* 2000, **22**:21-29.
63. Pohl RH, McClure P, De Rosa CT. **Persistent chemicals found in breast milk and their possible interactions.** *Environ Toxicol Pharmacol* 2004, **18**: 259-266.
64. WHO. **Fourth WHO-Coordinated survey of human milk for persistent organic pollutants in cooperation with UNEP: Guidelines for developing a national protocol.** World Health Organization, Food Safety, Foodborne diseases and Zoonoses Department. Geneva, Switzerland. 43p. 2007. Available at: <http://www.who.int/foodsafety/chem/POPprotocol.pdf>
65. Berlin CM, Crase BL, Furst P, LaKind JS, Moy G, Needham LL, Pugh LC, Tully MR. **Methodologic considerations for improving and facilitating human milk research.** *Journal of Toxicology and Environmental Health Part A: Current issues.* 2005, **68**: 1803-1824.
66. Wallace L, Buckley T, Pellizzari E, Gordon S. **Breath measurements as volatile organic compound biomarkers.** *Environ Health Perspect* 1996, **104**(Suppl 5):861-869.
67. Gordon SM, Wallace LA, Brinkman MC, Callahan PJ, Kenny DV. **Volatile organic compounds as breath biomarkers for active and passive smoking.** *Environ Health Perspect* 2002, **110**:689-698.

68. Weisel C, Yu R, Roy A, Georgopoulos P. **Biomarkers of environmental benzene exposure.** *Environ Health Perspect* 1996, **104**(Suppl 6):1141-1146.
69. Mutti A, Corradi M, Goldoni M, Vettori MV, Bernard A, Apostoli P. **Exhaled metallic elements and serum pneumoproteins in asymptomatic smokers and patients with COPD or asthma.** *Chest* 2006, **129**:1288-1297.
70. Goldoni M, Catalani S, De Palma G, Manini P, Acampa O, Corradi M, Begonzi R, Apostoli P, Mutti A. **Exhaled breath condensate as a suitable matrix to assess lung dose and effects in workers exposed to cobalt and tungsten.** *Environ Health Perspect* 2004, **112**:1293-1298.
71. Kharitonov SA, Barnes PJ. **Exhaled biomarkers.** *Chest* 2006, **130**:1541-1546.
72. Olivieri M, Talamini G, Corradi M, Perbellini L, Mutti A, Tantucci C, Malerba M. **Reference values for exhaled nitric oxide (reveno) study.** *Respir Res* 2006, **7**:94-99.
73. Jackson AS, Sandrini A, Campbell C, Chow S, Thomas PS, Yates DH. **Comparison of biomarkers in exhaled breath condensate and bronchoalveolar lavage.** *Am J Respir Crit Care Med* 2007, **175**:222-227.
74. Carraro S, Rezzi S, Reniero F, Heberger K, Giordano G, Zanconato S, Guillou C, Baraldi E. **Metabolomics applied to exhaled breath condensate in childhood Asthma.** *Am J Respir Crit Care Med* 2007, **175**:987-990.
75. Di Natale C, Macagnano A, Martinelli E, Paolesse R, D'Arcangelo G, Roscioni C, Finazzi-Agro A, D'Amico A. **Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors.** *Biosens Bioelectron* 2003, **18**:1209-1218.
76. Machado RF, Laskowski D, Deffenderfer O, Burch T, Zheng S, Mazzone PJ, Mekhall T, Jennings C, Stoller JK, Pyle J, Duncan J, Dwelk RA, Erzurum SC. **Detection of lung**

cancer by sensor array analyses of exhaled breath. *Am J Respir Crit Care Med* 2005, **171**:1286-1291.

77. **Hanson CW, Thaler ER. Electronic nose prediction of a clinical pneumonia score: Biosensors and microbes.** *Anesthesiology* 2005, **102**:63-68.

78. **Yu JB, Byun HG, So MS, Huh JS. Analysis of diabetic patient's breath with conducting polymer sensor array.** *Sensor Actuat B-Chem* 2005, **108**:305-308.

79. **Nieuwenhuijsen MJ, Toledano MB, Eaton NE, Fawell J, Elliot P. Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review.** *Occup Environ Med* 2000, **57**:73-85.

80. **Horvath I, Hunt J, Barnes PJ. Exhaled breath condensate: methodological recommendations and unresolved questions.** *Eur Respir J* 2005, **26**:523-48.

81. **Covaci A, Schepens P. Chromatographic Aspects of the analysis of selected persistent organochlorine pollutants in human hair.** *Chromatographia*. 2001, **53** (Suppl 1):S366-S371.

82. **Covaci A, Tutudaki M, Tsatsakis AM, Schepens P. Hair analysis: another approach for the assessment of human exposure to selected persistent organochlorine pollutants.** *Chemosphere* 2002, **46**:413-418.

83. **Schramm KW. Hair: A matrix for non-invasive biomonitoring of organic chemicals in mammals.** *Bull Environ Contam Toxicol* 1997, **59**:396-402.

84. **Zhang H, Chai ZF, Sun HB. Human hair as a potential biomonitor for assessing persistent organic pollutants.** *Environ Int* 2007, **33**:685-693.

85. **Legrand M, Passos CJS, Mergler D, Chan HM. Biomonitoring of mercury exposure with single human hair strand.** *Environ Sci Technol* 2005, **39**:4594-4598.

86. Sela H, Karpaz Z, Zoriy M, Pickhardt C, Becker JS. **Biomonitoring of hair samples by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).** *Int J Mass Spectrom* 2007, **261**:199-207.
87. Schramm KW, Kuettner T, Weber S, Lützke K. **Dioxin hair analyse as monitoring tool.** *Organohal Comp* 1991, **7**:235.
88. Schramm KW. **Hair – biomonitoring of organic pollutants.** *Chemosphere* 2008, **72**:1103-1111.
89. Schramm KW. **Biomonitoring ausgewählter organischer Chemikalien mit Haaren.** Herbert Utz, München, 1999.
90. Schramm KW, Kettrup A. **Hair analysis of PCDD/F and PCB: applications to human- and ecotoxicology.** *Organohal Comp* 1995, **26**:201.
91. Nakao T, Aozasa O, Ohta S, Miyata H. **Assessment of human exposure to PCDDs, PCDFs and Co-PCB using hair as a human pollution indicator sample I: development of analytical method for human hair and evaluation for exposure assessment.** *Chemosphere* 2002, **48**:885-896.
92. Stupar J, Dolinsek F, Erzen I. **Hair-Pb longitudinal profiles and blood-Pb in the population of young Slovenian males.** *Ecotoxicol Environ Safety* 2007, **68**:134-143.
93. Gosselin NH, Brunet RC, Carrier G, Bourchard M, Feeley M. **Reconstruction of methylmercury intakes in indigenous populations from biomarker data.** *J Expo Sci Environ Epidemiol* 2006, **16**:19-29.
94. Rashed M, Soltan M. **Animal hair as biological indicator for heavy metal pollution in urban and rural areas.** *Environ Monitor Assess* 2005, **110**:41-53.

95. D'Havé H, Scheirs J, Covaci A, Schepens P, Verhagen R, De Coen W. **Non-destructive pollution exposure assessment in the European hedgehog (*Erinaceus europaeus*): Hair as an indicator of endogenous organochlorine compound concentrations.** *Environ Toxicol Chem* 2006, **25**:158-167.
96. Jaspers VLB, Covaci A, Van den Steen E, Eens M. **Is external contamination with organic pollutants important for concentrations measured in bird feathers.** *Environ Int* 2007, **33**:766-772.
97. Kim SJ, Dix DJ, Thompson KE, Murrell RN, Schmid JE, Gallagher JE, Rockett JC. **Gene expression in head hair follicles plucked from men and women.** *Ann Clin Lab Sci* 2006, **36**:115-126.
98. Nakao T, Aozasa O, Ohta S, Miyata H. **Survey of human exposure to PCDDs, PCDFs, and coplanar PCBs using hair as an indicator.** *Arch Environ Contam Toxicol* 2005, **49**:124-130.
99. Altshul L, Covaci A, Hauser R. **The relationship between levels of PCBs and pesticides in human hair and blood: Preliminary results.** *Environ Health Perspect* 2004, **112**:1193-1199.
100. Mehra R, Juneja M. **Biological monitoring of lead and cadmium in human hair and nail and their correlations with biopsy materials, age and exposure.** *Indian J Biochem Biophys* 2004, **41**:53-56.
101. Ng DDK, Chan CH, Soo MT, Lee RSY. **Low-level chronic mercury exposure in children and adolescents: Meta-analysis.** *Pediatr Int* 2007, **49**:80-87.
102. ATSDR (Agency for Toxic Substances and Disease Registry). *Hair Analysis Panel Discussion: Exploring the State of the Science.* Atlanta, GA, 2001.

103. **Martinez v, Creus A, Venegas W, Arroyo A, Beck JP, Gebel TW, Surralles J, Marcos R. Evaluation of micronucleus induction in a Chilean population environmentally exposed to arsenic.** *Mutat Res – Gen Tox En* 2004, **564**:65-74.
104. **Slotnick MJ, Nriago JO, Johnson MM, Linder AM, Savoie KL, Jamil HJ, Hammad AS. Profiles of trace elements in toenails of Arab-Americans in the Detroit area, Michigan.** *Biol Trace Elem Res* 2005, **107**:113-126
105. **Gareri J, Lynn H, Handley M, Rao C, Koren G. Prevalence of fetal ethanol exposure in a regional population-based sample by meconium analysis of fatty acid, ethyl esters.** *Ther Drug Monit* 2008, **30**:239-245.
106. **Chan D, Caprara D, Blanchette P, Klein J, Koren G. Recent developments in meconium and hair testing methods for the confirmation of gestational exposures to alcohol and tobacco smoke.** *Clin Biochem* 2004, **37**:429-438.
107. **Gareri J, Klein J, Koren G. Drugs of abuse testing in meconium.** *Clin Chim Acta* 2006, **366**:101-111.
108. **Hong Z, Gunter M, Randow FFE. Meconium: A matrix reflecting potential fetal exposure to organochlorine pesticides and its metabolites.** *Ecotox Environ Safe* 2002, **51**:60-64.
109. **Whyatt RM, Barr DB. Measurement of organophosphate metabolites in postpartum meconium as a potential biomarker of prenatal exposure: A validation study.** *Environ Health Persp* 2001, **109**:417-420.
110. **Wessels D, Barr DB, Mendola P. Use of biomarkers to indicate exposure of children to organophosphate pesticides: Implications for a longitudinal study of children's environmental health.** *Environ Health Persp* 2003, **111**:1939-1946.

111. Ramirez GB, Cruz MCV, Pagulayan MS, Ostrea E, Dalisay C. **The Tagum Study I: Analysis and clinical correlates of mercury in maternal and cord blood, breast milk, meconium, and infant's hair.** *Pediatrics* 2000, **106**:774-781.
112. Ostrea EM, Morales V, Ngoumgna E, Prescilla R, Tan E, Hernandez E, Ramirez GB, Cifra HL, Manlapaz ML. **Prevalence of fetal exposure to environmental toxins as determined by meconium analysis.** *Neurotoxicology* 2002, **23**:329-339.
113. Turker G, Ergen K, Karakoc Y, Arisoy AE, Barutcu UB. **Concentrations of toxic metals and trace elements in the meconium of newborns from an industrial city.** *Biol Neonate* 2006, **89**:244-250.
114. Bergdahl IA, Skerfving S. **Biomonitoring of lead exposure-alternatives to blood.** *J Toxicol Env Heal A* 2008, **71**:1235-1243.
115. Yuan C, Lu X, Oro N, Wang Z, Xia Y, Wade TJ, Mumford J, Le XC. **Arsenic speciation analysis in human saliva.** *Clin Chem* 2008, **54**:163-171.
116. Nriagu J, Burt B, Linder A, Ismail A, Sohn W. **Lead levels in blood and saliva in a low-income population of Detroit, Michigan.** *Int J Hyg Enviro Heal* 2006, **209**:109-121.
117. Melchart D, Kohler W, Linde K, Zilker T, Kremers L, Saller R, Halbach S. **Biomonitoring of mercury in patients with complaints attributed to dental amalgam, healthy amalgam bearers, and amalgam-free subjects: A diagnostic study.** *Clin Toxicol* 2008, **46**:133-140.
118. Wang DX, Du XQ, Zheng W. **Alteration of saliva and serum concentrations of manganese, copper, zinc, cadmium and lead among career welders.** *Toxicol Lett* 2008, **176**:40-47.

119. Timchalk C, Campbell JA, Liu GD, Lin YH, Kousba AA. **Development of a non-invasive biomonitoring approach to determine exposure to the organophosphorus insecticide chlorpyrifos in rat saliva.** *Toxicol Appl Pharm* 2007, **219**:217-225.
120. Lu CS, Rodriguez T, Funez A, Irish RS, Fenske RA. **The assessment of occupational exposure to diazinon in Nicaraguan plantation workers using saliva biomonitoring.** *Ann NY Acad Sci* 2006, **1076**:355-365
121. Denovan LA, Lu C, Hines CJ, Fenske RA. **Saliva biomonitoring of atrazine exposure among herbicide applicators.** *Int Arch Occ Env Heal* 2000, **73**:457-462.
122. Silva MJ, Reidy JA, Samandar E, Herbert AR, Needham LL, Calafat AM. **Detection of phthalate metabolites in human saliva.** *Arch Toxicol* 2005, **79**:647-652
123. Sugita T, Kawamura Y, Tanimura M, Matsuda R, Niino T, Ishibashi T, Hirabahashi N, Matsuki Y, Yamada T, Maitani T. **Estimation of daily oral exposure to phthalates derived from soft polyvinyl chloride baby toys.** *Food Hyg Soc Jpn* 2003, **44**:96-102.
124. Marchesi JR, Holmes E, Khan F, Kochhar S, Scanlan P, Shanahan F, Wilson ID, Wang YL. **Rapid and noninvasive metabonomic characterization of inflammatory bowel disease.** *J Proteome Res* 2007, **6**:546-551.
125. Tonus C, Neupert G, Sellinger M. **Colorectal cancer screening by non-invasive metabolic biomarker fecal tumor M2-PK.** *World J Gastroentero* 2006, **12**:7007-7011.
126. Henn BD, McMaster S, Padilla S. **Measuring cholinesterase activity in human saliva.** *J Toxicol Env Heal A* 2006, **69**:1805-1818.
127. Wang J, Timchalk C, Lin YH. **Carbon nanotube-based electrochemical sensor for assay of salivary cholinesterase enzyme activity: An exposure biomarker of organophosphate and nerve agents.** *Environ Sci Technol* 2008, **42**:2688-2693

128. Bloching M, Stephan D, Agha-Mir-Salim P, Berghaus A, Lautenschlager C, Grummt T. **The Ames test as possible biomarker.** *HNO* 2001, **49**:400-446.
129. Bloching MB, Barnes J, Aust W, Knipping S, Neumann K, Grummt T, Naim R. **Saliva as a biomarker for head and neck squamous cell carcinoma: In vitro detection of cytotoxic effects by using the plating efficiency index.** *Oncol Rep* 2007, **18**:1551-1556.
130. Holland N, Bolognesi C, Kirsch-volders M, Bonassi S, Zeiger E, Knasmueller S, Fenech M. **The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps.** *Mutat Res – Rev Mutat* 2008, **659**:1-2.
131. Salama SA, Serrana M, Au WW. 1999. **Biomonitoring using accessible human cells for exposure and health risk assessment.** *Mutat Res* 1999, **436**:99-112.
132. Celik A, Cavas T, Ergene-Gozukara S. 2003. **Cytogenetic biomonitoring in petrol station attendants: micronucleus test in exfoliated buccal cells.** *Mutagenesis* 2003, **18**:417-421.
133. Chen C, Arjomandi M, Qin H, Balmes J, Tager I, Holland N. **Cytogenetic damage in buccal epithelia and peripheral lymphocytes of young healthy individuals exposed to ozone.** *Mutagenesis* 2006, **21**:131-137.
134. Moore LE, Warner ML, Smith AH, Kalman D, Smith MT. **Use of the fluorescence micronucleus assay to detect the genotoxic effect of radiation and arsenic exposure in human epithelial cells.** *Environ Mol Mutagen* 1996, **27**:176-184.
135. Slotnick MJ, Meliker JR, Kannan S, Nriagu JO. **Effects of nutritional measures on toenail arsenic concentration as a biomarker of arsenic exposure.** *Biomarkers* 2008, **13**:451-466.

136. **Were FH, Njue W, Murungi J, Wanjau R. Use of human nails as bio-indicators of heavy metals environmental exposure among school age children in Kenya. *Sci Total Environ* 2008, **393**:376-384.**
137. **Brima EL, Haris PI, Jenkins RO, Polya DA, Gault AG, Harrington CF. Understanding arsenic metabolism through a comparative study of arsenic levels in the urine, hair and fingernails of healthy volunteers from three unexposed ethnic groups in the United Kingdom. *Toxicol Appl Pharm* 2006, **216**:122-130.**
138. **Babalola OO, Ojo LO, Aderemi MO. Lead levels in some biological samples of auto-mechanics in Abeokuta, Nigeria. *Indian J Biochem Bio* 2005, **42**:401-403.**
139. **Ostrea EM, Bielaxski DM, Posecion NC, Corrion M, Villanueva-Uy E, Jin Y, Janisse JJ, Ager JW. A comparison of infant hair, cord blood and meconium analysis to detect fetal exposure to environmental pesticides. *Environ Res* 2008, **106**:277-283.**
140. **Oral R, Strang T. Neonatal illicit drug screening practices in Iowa: The impact of utilization of a structured screening protocol. *J Perinat* 2006, **26**:660-666**
141. **Moore CM . *Drugs-of-abuse in meconium specimens. In: Jenkins AJ. Forensic science and medicine: Drug testing in Alternate Biological Specimens.* Humana Press, Totowa, NJ. USA; 2008.**
142. **Whyatt RM, Barr DB. Measurement of organophosphate metabolites in postpartum meconium as a potential biomarker of prenatal exposure: A validation study. *Environ Health Persp* 2001, **109**:417-420.**
143. **Van Leeuwen FXR, Malisch R. Results of the third round of the WHO coordinated exposure study on the levels of PCBs, PCDDs and PCDFs in human milk. *Organohal Comp* 2002, **56**:311-316.**

144. **Shin HS, Kim JG, Shin YJ, Jee SH. Sensitive and simple method for the determination of nicotine and cotinine in human urine, plasma and saliva by gas chromatography-mass spectrometry. *J Chromatogr B*, 2002, 769:177-183.**
145. **Becker AB, Manfreda J, Ferguson AC, Dimich-Ward H, Watson WTA, Chan-Yeung M. Breast-feeding and environmental tobacco smoke exposure. *Arch Pediatr Adolesc Med* 1999, 153:689-691.**
146. **Suzuki G, Nakano M, Nakano S. Distribution of PCDD/PCDFs and Co-PCBs in human maternal blood, cord blood, milk and adipose tissue: Dioxins showing high toxic equivalency factor accumulate in the placenta. *Biosci Biotechnol Biochem* 2005, 69:1836-1847.**
147. **Osman K, Akesson A, Berglund M, Bremme K, Schutz A, Ask K, Vather M. Toxic and essential elements in placentas of Swedish women. *Clin Biochem* 2000, 33:131-138.**
148. **Hall M, Gamble M, Slavkovich V, Liu XH, Levy D, Cheng ZQ, Van Geen A, Yunus M, Rahman M, Pisner JR, Graziano J. Determinants of arsenic metabolism: blood arsenic metabolites, plasma folate, cobalamin, and homocysteine concentrations in maternal-newborn pairs. *Environ Health Perspect* 2007, 115:1503-1509.**
149. **Ramon R, Murcia M, Ballester F, Rebagliato M, Lacasana M, Vioque J, Llop S, Amurrio A, Aguinagalde X, Marco A, Leon G, Ibarluzea J, Ribas-Fito N. Prenatal exposure to mercury in a prospective mother-infant cohort study in a Mediterranean area, Valencia, Spain. *Sci Total Environ* 2008, 392:69-78.**
150. **Becker K, Kaus S, Krause C, Lepom P, Schulz C, Seiwert M, Seifert B. Umwelt-Survey 1998, Band III: Human-Biomonitoring. Stoffgehalte in Blut und Urin der Bevölkerung in Deutschland(German Environmental Survey 1998, Vol. III: Human Biomonitoring. Pollutants in Blood and Urine of the German Population), 1998.**

151. Pichini S, Basagana X, Pacifici R, Garcia O, Puig C, Vall O, Harris J, Zuccaro P, Segura J, Sunyer J. **Cord serum cotinine as a biomarker of fetal exposure to cigarette smoke at the end of pregnancy.** *Environ Health Perspect* 2000, **108**:1079-1083.
152. Heitland P, Koster HD. **Biomonitoring of 30 trace elements in urine of children and adults by ICP-MS.** *Clin Chim Acta* 2006, **365**:310-318.
153. **National Resources Defense Council** [<http://www.nrdc.org/breastmilk/lead.asp>]
154. Krause C, Babisch W, Becker K, Bernigau W, Hoffmann K, Nöllke P, Schulz C, Schwabe R, Seiwert S, Thefeld W. **Umwelt-Survey 1990/92, Band Ia: Studienbeschreibung und Human-Biomonitoring: Deskription der Spurenelementgehalte in Blut und Urin der Bevölkerung der Bundesrepublik Deutschland.** (Environmental Survey 1990/92, Vol. Ia: Study Design and Human Biomonitoring, Trace Elements in Blood and Urine of the German Population), 1996.
155. McDowell MA, Dillon CF, Osterloh J, Bolger PM, Pellizzari E, Fernando R, de Oca RM, Schober SE, Sijks T, Jones RL, Mahaffey KR. **Hair mercury levels in US children and women of childbearing age: Reference range data from NHANES 1999-2000.** *Environ Health Perspect* 2004, **112**:1165-1171.
156. ATSDR (Agency for Toxic Substances and Disease Registry). *Toxicological Profile for Arsenic. August 2007.* Agency for Toxic Substances & Disease Registry. Department of Health and Human Services. Atlanta, GA. USA, 2007.
157. Shen H, Maine KM, Andersson AM, Damgaard IN, Virtanen HE, Skakkebaek NE, Toppari J, Schramm KW. **Concentrations of persistent Organochlorine compounds in human milk and placenta are higher in Denmark than in Finland.** *Hum Reprod* 2008, **23**:201-210.

158. Ribas-Fito N, Torrent M, Carrizo D, Julvez J, Grimalt JO, Sunyer J. **Exposure to hexachlorobenzene during pregnancy and children's social behavior at 4 years of age.** *Environ Health Perspect* 2007, **115**:447-450.
159. To-Figueras J, Barrot C, Sala M, Otero R, Sivla M, Ozalla MD, Herrero C, Corbella J, Grimalt J, Sunyer J. **Excretion of hexachlorobenzene and metabolites in feces in a highly exposed human population.** *Environ Health Perspect* 2000, **108**:595-598.
160. Van Leeuwen FXR, Malisch R. **Results of the third round of the WHO coordinated exposure study on the levels of PCBs, PCDDs and PCDFs in human milk.** *Organohal Comp* 2002, **56**:311-316.
161. Shin HS, Kim JG, Shin YJ, Jee SH. **Sensitive and simple method for the determination of nicotine and cotinine in human urine, plasma and saliva by gas chromatography-mass spectrometry.** *J Chromatogr B*, 2002, **769**:177-183.
162. Becker AB, Manfreda J, Ferguson AC, Dimich-Ward H, Watson WTA, Chan-Yeung M. **Breast-feeding and environmental tobacco smoke exposure.** *Arch Pediatr Adolesc Med* 1999, **153**:689-691.

Additional files provided with this submission:

Additional file 1: table 1.doc, 37K

<http://www.ehjournal.net/imedia/2548439932316260/supp1.doc>

Additional file 2: table 2.doc, 39K

<http://www.ehjournal.net/imedia/2447571162316261/supp2.doc>

Additional file 3: table 3.doc, 36K

<http://www.ehjournal.net/imedia/1008805754231626/supp3.doc>