international journal of andrology, (2005) doi: 10.1111/j.1365-2605.2005.00570.x

REVIEW

Human exposure assessment to environmental chemicals using biomonitoring

ANTONIA M. CALAFAT, XIAOYUN YE, MANORI J. SILVA, ZSUZSANNA KUKLENYIK AND LARRY L. NEEDHAM

Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

Summary

In modern societies, humans may be exposed to a wide spectrum of environmental chemicals. Although the health significance of this exposure for many chemicals is unknown, studies to investigate the prevalence of exposure are warranted because of the chemicals' potential harmful health effects, as often indicated in animal studies. Three tools have been used to assess exposure: exposure history/questionnaire information, environmental monitoring, and biomonitoring (i.e. measuring concentrations of the chemicals, their metabolites, or their adducts in human specimens). We present an overview on the use of biomonitoring in exposure assessment using phthalates, bisphenol A and other environmental phenols, and perfluorinated chemicals as examples. We discuss some factors relevant for interpreting and understanding biomonitoring data, including selection of both biomarkers of exposure and human matrices, and toxicokinetic information. The use of biomonitoring in human risk assessment is not discussed.

Keywords: biological monitoring, emerging pollutants, glucuronidation, matrix, NHANES, oxidative metabolism, perfluorinated chemicals, phenol, phthalates

Introduction

The probability of non-occupational human exposure to chemicals present in commonly used products is high given their high production volumes and extensive use. For the most part, no information exists about the extent of human exposure to these chemicals, and the potential toxic health effects of these compounds in humans are largely unknown. As information on risk to human health from exposure to environmental chemicals is limited, studies to investigate the prevalence of these exposures are warranted. However, exposure assessment is complex in epidemiological studies because human exposure does not occur under controlled conditions of dose–response evaluations associated with animal studies (exposure-health effect). Indirect measures of exposure that combine environmental monitoring and exposure history/questionnaire data have been used to assess human exposure to environmental chemicals. Advances in analytical chemistry have made measuring trace levels of multiple environmental chemicals in biological tissues (i.e. biological monitoring or biomonitoring) possible and have contributed to increased use of biomonitoring in exposure assessment (Pirkle *et al.*, 1995). Although biomonitoring can also be used in risk assessment, assessing potential health risks from biomonitoring exposure data will not be discussed in this overview.

Correspondence: Antonia M. Calafat, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy., NE, Mailstop F17, Atlanta, GA 30341, USA. E-mail: acalafat@cdc.gov

Biomonitoring programmes

Biomonitoring programmes are useful for investigating human exposure to environmental chemicals. One of these programmes, the National Health and Nutrition Examination Survey (NHANES), conducted annually in the United States by the Centers for Disease Control and Prevention (CDC) is designed to collect data on the health and nutritional status of the non-institutionalized, civilian US population (CDC, 2003a). The survey includes a physical examination, and collection of detailed medical history and biological specimens from participants. Although biological specimens are used mostly for clinical and nutritional testing, some can be used to assess exposure to environmental chemicals. Beginning with NHANES 1999, levels of selected chemicals in urine and blood of NHANES participants have been reported in the National Report on Human Exposure to Environmental Chemicals (CDC, 2003b). These reports provide the most comprehensive biomonitoring assessment of the US population's exposure to environmental chemicals, and may help prioritize and foster research on human health risks that result from exposure, largely unknown for many of the chemicals included in the reports.

The NHANES data can be used to establish reference ranges for selected chemicals, provide exposure data for risk assessment (e.g. set intervention and research priorities, evaluate effectiveness of public health measures), and monitor exposure trends. Reference ranges can be used to assist epidemiological investigations, to correlate the levels to other NHANES parameters/measurements (including potential health effects), and to identify (i) populations with the highest exposures, (ii) potential sources/routes of exposure, and (iii) chemicals with highest prevalence/frequency (Pirkle *et al.*, 1995). However, even a comprehensive programme such as NHANES has limitations: persons under 1 year of age are not included, and no data are collected on foetal exposures. Furthermore, NHANES by design does not intentionally include population groups that might be highly exposed to various point sources and could be examined to evaluate possible associations between high exposures and adverse health effects.

Analytical considerations for biomonitoring

Blood (or its components) and urine are the most common matrices for biomonitoring; many alternative matrices can also be used (Needham *et al.*, 2005a). Some are particularly useful for assessing exposure during foetal and early childhood life, when humans are most susceptible to potential adverse health effects of environmental chemicals. Amniotic fluid, cord blood and meconium are promising matrices for monitoring prenatal exposures (Burse *et al.*, 2000; Foster *et al.*, 2000; Whyatt & Barr, 2001). Breast milk can be used to monitor neonatal exposures and its analysis also can provide an estimate of foetal exposures to some chemicals (Landrigan *et al.*, 2002; LaKind *et al.*, 2004). Biological matrices are complex, can be difficult to obtain, and may be available only in small amounts. Furthermore, although environmental chemicals are normally present in the matrix at trace levels, other matrix components occur at higher concentrations. Therefore, highly sensitive, specific and selective multianalyte methods for the extraction, separation and quantification of these chemicals must be developed (Needham *et al.*, 2005b).

Phthalates are widely used as plasticizers, in consumer goods, and in personal care products. Prenatal exposure to some phthalates produced developmental and reproductive toxicity in experimental animals, particularly in male offspring (Gray *et al.*, 2000; Mylchreest *et al.*, 2000; Ema *et al.*, 2003). Currently, evidence on the association between exposure to phthalates and human health effects is limited. Phthalates are non-persistent compounds that are rapidly metabolized and excreted (Fig. 1) (ATSDR, 1995, 1997, 2001, 2002). Many are ubiquitous, and their direct measurements in biological specimens are subject to error because of contamination that can occur during sample collection, storage and throughout the analytical measurement process. To minimize contamination, the preferred biomonitoring approach is to measure urinary levels of phthalate monoester metabolites (Blount *et al.*, 2000).

Phthalates can be hydrolysed to their hydrolytic monoesters by esterases present in milk (Calafat *et al.*, 2004) and serum (Kato *et al.*, 2003); other matrices such as amniotic fluid and meconium may also contain esterases. If the concentration of hydrolytic monoesters in matrices other than urine is used to estimate exposure, care must be taken to minimize contamination with phthalates during sampling, storage and analysis. Otherwise, measured concentrations may include an unknown contribution from hydrolysis of

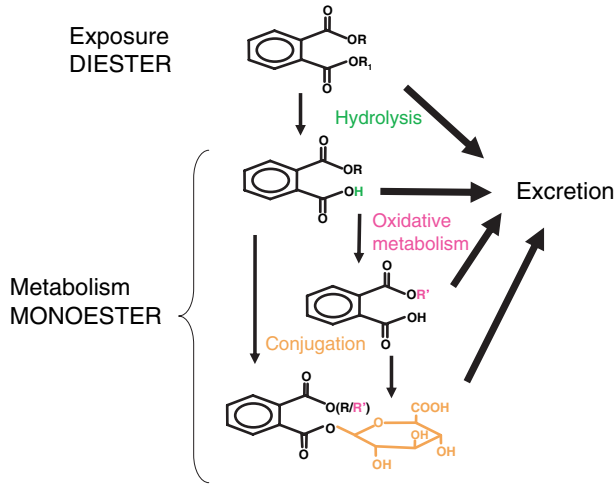

Figure 1. Phthalates metabolize to hydrolytic monoesters and may be further metabolized to oxidative products. Oxidative metabolism is prevalent for high molecular weight phthalates. Phthalate metabolites can be excreted unchanged or as conjugated species after undergoing phase II biotransformation.

contaminant phthalates by endogenous esterases. Therefore, not only the chemical properties of the compounds of interest, but also the composition of the matrix and its potential effects on concentrations of selected analytes must be considered when developing sampling, storage and analysis protocols for biomonitoring studies.

Distribution of environmental chemicals in the body

After exposure, environmental chemicals may enter the body, and once they reach the blood systemic circulation, they can be distributed into various body compartments, where they can be in equilibrium with blood concentrations, secretion concentrations (e.g. milk), or both. To compare concentrations in blood and other matrices, information on partitioning of these chemicals from blood into tissues is needed (Needham *et al.*, 2005a).

Traditional persistent lipophilic compounds can partition from blood into adipose tissue and may be found in milk for lactating women (Solomon & Weiss, 2002). By contrast, the high affinity for serum proteins of perfluorochemicals (PFCs) (Jones *et al.*, 2003), a class of persistent compounds used since the 1950s in commercial applications including surfactants, lubricants, paper and textile coatings, polishes, food packaging, and fire-retarding foams, makes serum the preferred matrix of choice for monitoring human exposure to PFCs (Hansen *et al.*, 2001). However, in Sprague–Dawley rats dosed with perfluorooctane sulfonate (PFOS) through gestation, PFOS was detected in the serum and milk (Kuklenyik *et al.*, 2004) suggesting that PFOS may partition into milk, although serum concentrations in the dosed rats were more than 4000 times higher than the median concentration of PFOS in selected populations in the United States (Olsen *et al.*, 2005). Non-persistent chemicals can also be found in milk (Otaka *et al.*, 2003; Calafat *et al.*, 2004; Sun *et al.*, 2004). Bisphenol A (BPA), used to manufacture polycarbonate plastics and epoxy resins, and ortho-phenyl phenol (OPP), a biocide, have been found in urine (CDC, 2003b; Calafat *et al.*, 2005). We also detected BPA and OPP in human milk (Fig. 2), suggesting that breast milk maybe a route of exposure to BPA and OPP for breastfeeding infants. Although PFCs, BPA and OPP have shown several toxicities in laboratory animals, the human health effects of these compounds at environmental exposure levels are not known.

Selection of biomarkers of exposure

Low molecular weight phthalates mostly metabolize to their hydrolytic monoesters (ATSDR, 1995, 2001). High molecular weight phthalates, such as those with eight or more carbons in the alkyl chain (e.g. di[2-ethylhexyl] phthalate, DEHP), metabolize to their hydrolytic monoesters which can be further transformed to oxidative products (ATSDR, 1997, 2002; McKee *et al.*, 2002; Koch *et al.*, 2004a). Oxidative metabolites are more water soluble than the corresponding hydrolytic monoesters, explaining the higher urinary levels in human populations of oxidative

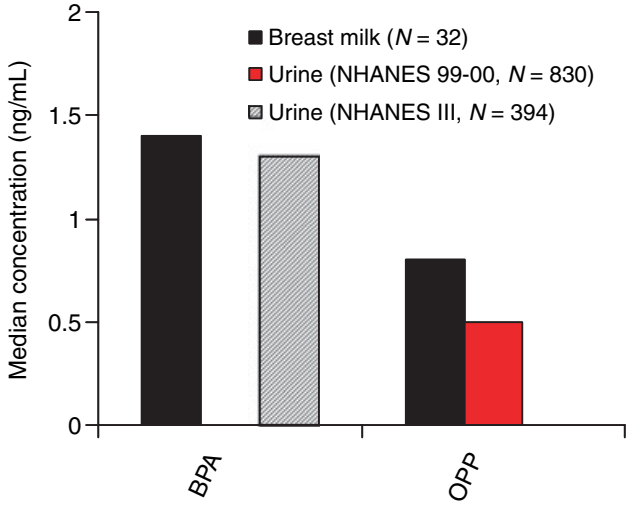

Figure 2. After exposure, environmental chemicals may distribute into various body fluids or tissues. Concentrations of bisphenol A (BPA) and ortho-phenyl phenol (OPP) in milk from 32 lactating women, and in urine samples collected from participants of the Third National Health and Nutrition Examination Survey (NHANES III) and of NHANES 1999–2000.

metabolites than of hydrolytic monoesters for a given phthalate (Barr *et al.*, 2003; Koch *et al.*, 2003; Kato *et al.*, 2004; Koch *et al.*, 2004b). Using hydrolytic monoesters of some high molecular weight phthalates as biomarkers of exposure may allow exposure comparison with the parent phthalate among studies. However, using these metabolites as sole biomarkers to compare relative exposures to various phthalates can be misleading especially when comparing monoester concentrations of high and low molecular weight phthalates, because metabolism of the former results in more metabolites, thus decreasing the relative amounts of their hydrolytic monoester metabolites. To date, research on the oxidative metabolism of phthalates has been largely limited to phthalates with a defined chemical composition (e.g. DEHP). Most high molecular weight phthalates are complex mixtures of isomers (e.g. di-isononyl phthalate); their composition varies depending on the nature of the mixture of alcohols used for their synthesis, which, in turn, may vary upon manufacturers. Metabolism of isomeric high molecular weight phthalates will result in multiple hydrolytic and oxidative monoesters. In rats, hydrolytic monoesters represent a very small percentage of the phthalate dose (McKee *et al.*, 2002). Although metabolic differences among species are possible, oxidative metabolites are likely to be the most abundant urinary metabolites of isomeric high molecular weight phthalates in humans. To properly assess the prevalence of exposure to these phthalates, research needs to focus on identifying and characterizing suitable oxidative metabolites. Until then, exposure to isomeric high molecular weight phthalates will likely be underestimated.

Oxidative metabolism may have contributed, at least in part, to the relatively low urinary levels and frequency of detection of 4-nonyl phenol (NP) in a group of US adults

(Calafat *et al.*, 2005). If oxidative metabolism of NP prevails in humans, like in animals, oxidative metabolites of NP may be better biomarkers to assess exposure to NP than NP itself. Moreover, 4-n-NP, the measured NP isomer, represents a small percentage of the NP used in commercial mixtures. Exposure to NP may be underestimated unless the most appropriate urinary biomarker(s) is used.

In summary, biomonitoring provides a reliable estimate of internal dose. Comprehensive biomonitoring programmes, such as NHANES, must continue. However, understanding of toxicokinetics of the environmental chemicals (e.g. distribution among body compartments, metabolism) and of their bioactivity at environmental exposure levels are required to properly interpret biomonitoring measurements. Age; diet; route, frequency, and magnitude of exposure; potential synergistic or antagonistic interactions among chemicals; and genetic factors, among others, are critical in determining health outcomes associated with exposure to environmental chemicals. As biomonitoring provides an integrated measure of exposure from all sources and routes, adequate sampling and storage protocols, and validated analytical methods that take into account both the nature of the matrix and biomarkers must be used. For relatively lipophilic non-persistent compounds (e.g. phthalates, alkyl phenols), formation of oxidative metabolites may be critical for facilitating their urinary excretion. To maximize the impact of biomonitoring in public health, future research should focus on (i) identifying the compounds best suited for use as biomarkers, in particular those that may provide the greatest analytical sensitivity (e.g. oxidative metabolites), (ii) characterizing their potential bioactivity in humans, (iii) improving the understanding of their toxicokinetics in different populations and with different doses with emphasis on foetal and neonatal exposures, when susceptibility to potential adverse health effects of environmental chemicals may be highest, and (iv) studying targeted populations with known source(s) of exposure to facilitate relating internal exposure to potential health effects.

References

ATSDR (1995) *Toxicological Profile for Diethyl Phthalate (DEP)*. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (1997) *Toxicological Profile for Di-n-octyl Phthalate (DNOP)*. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (2001) *Toxicological Profile for Di-n-butyl Phthalate (DBP)*. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR (2002) *Toxicological Profile for Di(2-ethylhexyl)phthalate (DEHP)*. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Barr, D. B., Silva, M. J., Kato, K., Reidy, J. A., Malek, N. A., Hurtz, D., Sadowski, M., Needham, L. L., Calafat, A. M. (2003) Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. *Environmental Health Perspectives* **111**, 1148–1151.

Blount, B. C., Milgram, K. E., Silva, M. J., Malek, N. A., Reidy, J. A., Needham, L. L., Brock, J. W. (2000) Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS. *Analytical Chemistry* **72**, 4127–4134.

Burse, V. W., Najam, A. R., Williams, C. C., Korver, M. P., Smith, B. F., Jr, Sam, P. M., Young, S. L., Needham, L. L. (2000) Utilization of umbilical cords to assess in utero exposure to persistent pesticides and polychlorinated biphenyls. *Journal of Exposure Analysis & Environmental Epidemiology* **10**, 776–788.

Calafat, A. M., Slakman, A. R., Silva, M. J., Herbert, A. R., Needham, L. L. (2004) Automated solid phase extraction and quantitative analysis of human milk for 13 phthalate metabolites. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* **805**, 49–56.

Calafat, A. M., Kuklenyik, Z., Reidy, J. A., Caudill, S. P., Ekong, J., Needham, L. L. (2005) Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environmental Health Perspectives* **113**, 391–395.

CDC (2003a) *National Health and Nutrition Examination Survey*. National Center for Health Statistics. (http://www.cdc.gov/nchs/nhanes.htm)

CDC (2003b) *Second National Report on Human Exposure to Environmental Chemicals*. Centers for Disease Control and Prevention; National Center for Environmental Health; Division of Laboratory Sciences, Atlanta, GA. (http://www.cdc.gov/exposurereport)

Ema, M., Miyawaki, E., Hirose, A., Kamata, E. (2003) Decreased anogenital distance and increased incidence of undescended testes in fetuses of rats given monobenzyl phthalate, a major metabolite of butyl benzyl phthalate. *Reproductive Toxicology* **17**, 407–412.

Foster, W., Chan, S., Platt, L., Hughes, C. (2000) Detection of endocrine disrupting chemicals in samples of second trimester human amniotic fluid. *Journal of Clinical Endocrinology and Metabolism* **85**, 2954–2957.

Gray, L. E., Ostby, J., Furr, J., Price, M., Veeramachaneni, D. N. R., Parks, L. (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicological Sciences* **58**, 350–365.

Hansen, K. J., Clemen, L. A., Ellefson, M. E., Johnson, H. O. (2001) Compound-specific, quantitative characterization of organic: Fluorochemicals in biological matrices. *Environmental Science & Technology* **35**, 766–770.

Jones, P. D., Hu, W. Y., De Coen, W., Newsted, J. L., Giesy, J. P. (2003) Binding of perfluorinated fatty acids to serum proteins. *Environmental Toxicology and Chemistry* **22**, 2639–2649.

Kato, K., Silva, M. J., Brock, J. W., Reidy, J. A., Malek, N. A., Hodge, C. C., Nakazawa, H., Needham, L. L., Barr, D. B. (2003) Quantitative detection of nine phthalate metabolites in human serum using reversed-phase high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of Analytical Toxicology* **27**, 284–289.

Kato, K., Silva, M. J., Reidy, J. A., Hurtz, D., Malek, N. A., Needham, L. L., Nakazawa, H., Barr, D. B., Calafat, A. M. (2004) Mono(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethylhexyl) phthalate. *Environmental Health Perspectives* **112**, 327–330.

Koch, H. M., Rossbach, B., Drexler, H., Angerer, J. (2003) Internal exposure of the general population to DEHP and other

phthalates – determination of secondary and primary phthalate monoester metabolites in urine. *Environmental Research* **93**, 177–185.

Koch, H. M., Bolt, H. M., Angerer, J. (2004a) Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. *Archives of Toxicology* **78**, 123–130.

Koch, H. M., Drexler, H., Angerer, J. (2004b) Internal exposure of nursery-school children and their parents and teachers to di(2-ethylhexyl)phthalate (DEHP). *International Journal of Hygiene and Environmental Health* **207**, 15–22.

Kuklenyik, Z., Reich, J. A., Tully, J. S., Needham, L. L., Calafat, A. M. (2004) Automated solid-phase extraction and measurement of perfluorinated organic acids and amides in human serum and milk. *Environmental Science & Technology* **38**, 3698–3704.

LaKind, J. S., Wilkins, A. A., Berlin, C. M., Jr (2004) Environmental chemicals in human milk: a review of levels, infant exposures and health, and guidance for future research. *Toxicology and Applied Pharmacology* **198**, 184–208.

Landrigan, P. J., Sonawane, B., Mattison, D., McCally, M., Garg, A. (2002) Chemical contaminants in breast milk and their impacts on children's health: An overview. *Environmental Health Perspectives* **110**, A313–A315.

McKee, R. H., El Hawari, M., Stoltz, M., Pallas, F., Lington, A. W. (2002) Absorption, disposition and metabolism of Di-isononyl phthalate (DINP) in F-344 rats. *Journal of Applied Toxicology* **22**, 293–302.

Mylchreest, E., Wallace, D. G., Cattley, R. C., Foster, P. M. D. (2000) Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(n-butyl) phthalate during late gestation. *Toxicological Sciences* **55**, 143–151.

Needham, LL, Barr, DB, Calafat, AM (2005a) Characterizing children's exposures: beyond NHANES. *Neurotoxicology* **26**, 547–553.

Needham, L. L., Patterson, D. G., Barr, D. B., Grainger, J., Calafat, A. M. (2005b) Uses of speciation techniques in biomonitoring for assessing human exposure to organic environmental chemicals. *Analytical and Bioanalytical Chemistry* **381**, 397–404.

Olsen, G. W., Huang, H. Y., Helzlsouer, K. J., Hansen, K. J., Butenhoff, J. L., Mandel, J. H. (2005) Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. *Environmental Health Perspectives* **113**, 539–545.

Otaka, H., Yasuhara, A., Morita, M. (2003) Determination of bisphenol A and 4-nonylphenol in human milk using alkaline digestion and cleanup by solid-phase extraction. *Analytical Sciences* **19**, 1663–1666.

Pirkle, J. L., Needham, L. L., Sexton, K. (1995) Improving exposure assessment by monitoring human tissues for toxic chemicals. *Journal of Exposure Analysis & Environmental Epidemiology* **5**, 405–424.

Solomon, G. M., Weiss, P. M. (2002) Chemical contaminants in breast milk: time trends and regional variability. *Environmental Health Perspectives* **110**, A339–A347.

Sun, Y., Irie, M., Kishikawa, N., Wada, M., Kuroda, N., Nakashima, K. (2004) Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. *Biomedical Chromatography* **18**, 501–507.

Whyatt, R. M., Barr, D. B. (2001) Measurement of organophosphate metabolites in postpartum meconium as a potential biomarker of prenatal exposure: a validation study. *Environmental Health Perspectives* **109**, 417–420.

Received 3 June 2005; revised 5 July 2005; accepted 11 July 2005