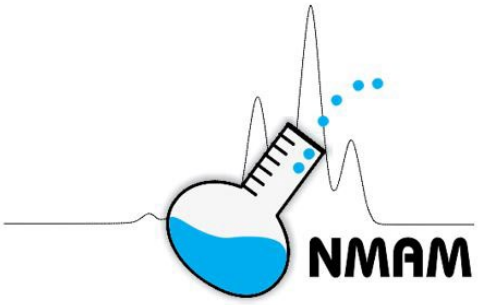

NIOSH Manual of Analytical Methods (NMAM), 5th Edition

Application of Biological Monitoring Methods for Chemical Exposures in Occupational Health

by D. Gayle DeBord, Ph.D., Dale Shoemaker, Ph.D., Clayton B'Hymer, Ph.D., and John Snawder, Ph.D., DABT, NIOSH (retired)

This chapter is a revision and expansion of Chapter F: Application of Biological Monitoring Methods published in the 4th edition of the NIOSH Manual of Analytical Methods (NMAM) [NIOSH 1998]. Additional information has been incorporated and terminology has been updated to reflect the most current practice. A lengthy section on biomonitoring analytical method validation has been added.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

1 Introduction

Exposure assessment is a critical part of occupational health studies and risk mitigation activities. Biomonitoring can be an essential tool to determine exposures and/or health effects resulting from those exposures. According to a National Research Council (NRC) report [NRC 2006], biomonitoring can provide an efficient means to measure exposures and, when used with other information derived from toxicological, epidemiologic or modeling studies, can estimate the absorption into the body (dose), the influence of an exposure to an individual, and the potential health risks. Large population studies that incorporate biomonitoring efforts can identify chemicals found in the environment (including the occupational environment) and monitor the trends and distribution of exposures in the general population [CDC 2021, 2022].

Environmental measurements involving various media (such as air, water, soil, food, or surfaces) can detect the presence of hazards and sometimes predict individual exposure; for example, air monitoring predicts effects when the lung is the target organ. However, to evaluate biological, physical, or chemical agents that have been absorbed into the body, the magnitude of the absorbed dose and its contribution to total body burden, the measurement of biomarkers (biomonitoring) is required. Exposures for which biomarkers can be useful include the full range of occupational hazards from noise to stress to chemicals. While this document generally focuses on chemical exposures, considerations pertaining to purpose (objectives), study design criteria, quality assurance, ethics, and safety have general application. The guidance provided in this document is particularly relevant to urine and blood biomonitoring and is generally relevant to other biomonitoring matrices and assays in which response or susceptibility factors such as genetic markers or gene variants are measured. This document generally focuses on the utility of biomonitoring as a research tool. Only a few examples that are cited in this chapter are relevant to occupational health practice.

2 Applications of biomonitoring

Biomonitoring has many benefits. Adapted from Decker et al. [2013], biomonitoring benefits can include the following:

- Measure actual body burden
- Augment other exposure monitoring tools
- Capture all exposure routes, including dermal
- Detect unexpected exposures or routes of exposure
- Evaluate the effectiveness of control measures, including personal protective equipment (PPE)
- Provide biomarkers of potential health risks
- Use to reconstruct exposures following acute or accidental events if appropriate biomarkers are available

- Enhance individual or group risk assessments
- Provide valuable information for risk communication

Biomonitoring can enhance exposure assessment and provide information about health outcomes, depending upon the selected biomarker(s).

3 Categories of biomarkers

The National Academy of Sciences has defined biomarkers as measurable indicators in a biological system or organism. Examples include the presence of a chemical or its metabolite within biological specimens, measured alterations in structure or function, or identifiable genetic variations (Figure 1) [NRC 1987]. Biomarkers can be classified into three categories: exposure, effect or response, and susceptibility (Table 1) [NRC 1987]. While these categories can overlap, generally biomarkers of exposure provide a measure of body burden of a chemical or its metabolite. Susceptibility biomarkers estimate the impact that individual factors can have on exposure, uptake metabolism, and/or repair.

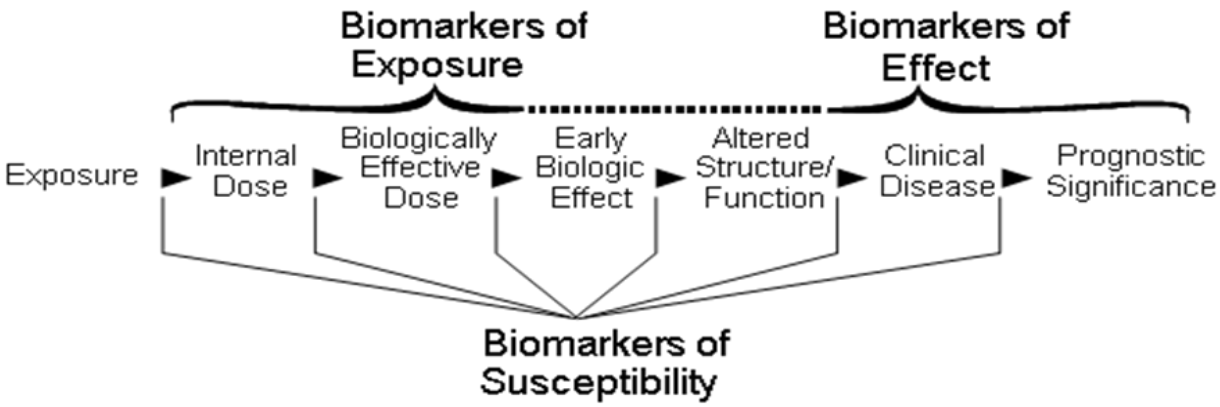

Figure 1 Continuum from exposure to disease. [Adapted from NRC 1987 and Schulte and Perera 1993]

Table 1. The three types of biomarkers

Type of Biomarker	Characteristics	Example
Exposure	Measurement that reflects absorption of a chemical into the body	Urine or blood concentration of chemical or metabolite
Effect	Biochemical, physiological, or other alteration that can be measured, which may or may not indicate a health impairment [WHO 2001]	DNA mutation or cytogenetic change
Susceptibility	Inherent or acquired sensitivities or resistance in response to specific exposures	Genetic polymorphisms in metabolic activation/deactivation enzymes

[Adapted from DeBord et al. 2015]

a. Biomarkers of exposure

Occupational exposure occurs mainly by inhalation or through the skin [NIOSH 1998] and less often by ingestion. The route of exposure may affect the internal dose and the biologically effective dose, which is the dose that interacts with the target organ or tissue. Biomarker measurements aggregate exposure across all pathways and all agents. While this aggregation is often advantageous, biomarker measurements cannot be used by themselves to determine the route(s) of exposure and in certain cases, the specific exposure agent. Examples of biomarkers of exposure include blood lead [Sexton et al. 2004], urinary cadmium [Menke et al. 2009], and serum dioxin [Manh et al. 2014]. Biomarkers of exposure indicate that an individual or population has been exposed and absorbed an agent into the body. Exposure biomarkers may or may not reflect future disease or health risk. Exposure concentration, exposure duration, route of exposure, pharmacokinetics of the chemical as it affects the distribution, metabolism and elimination, timing of sample collection, physiological variations in hydration status, and urinary flow can influence biomarker levels [Aylward et al. 2012; Aylward et al. 2014]. Individual characteristics such as gene variants, exertion, and co-exposures can also modify biomarker levels through the modification of absorption or metabolism.

b. Biomarkers of effect

Biomarkers of effect (also known as biomarkers of response) measure effects or responses in the body to an exposure. These changes may be early precursors of disease, specific clinical changes or markers for preclinical effects, or not be related to any specific health outcome. Biomarkers of effect may result in a measurable change in a pathway, macromolecule (DNA, RNA, protein), or other biomolecules. These changes may be reversible or permanent. An example of a biomarker of effect is the decreased levels of the enzyme acetylcholinesterase (AChE) in red blood cells (RBCs) of persons exposed to organophosphate (OP) pesticides [Richards et al. 1978]. Biomonitoring results of blood AChE levels can be used to determine (or confirm) exposure and to assess potential health risks and treatment needs [Richards et al. 1978]. A continuum exists across the biomarker spectrum. At low levels, a biomarker may indicate an exposure or an effect. However, as the exposure continues or increases, the biomarker level also increases, leading to a response or action level that needs intervention before health is permanently impacted. While biomarkers of exposure tend to be specific for a particular chemical or agent, biomarkers of effect are less specific and may show the effect of exposure to multiple chemicals or agents. For example, measuring blood AChE levels provides evidence of exposure to OP pesticides, but does not identify the specific pesticide responsible for the decrease in AChE. Similarly, carboxyhemoglobin levels increase after exposure to both carbon monoxide and methylene chloride, which is metabolized to carbon monoxide [Andersen et al. 1991].

c. Biomarkers of susceptibility

Biomarkers of susceptibility indicate whether an individual may be at increased or decreased risk for developing a disease after an exposure has occurred. Susceptibility biomarkers may also identify individuals whose body burden may be increased or decreased relative to other individuals because of differences in absorption, metabolism, or other biological processes. Biomarkers of susceptibility could include other exposures from the environment, current health status, or genetic traits, such as the activity of metabolizing enzymes. For example, the activity of CYP2E1, a cytochrome P450 metabolic enzyme, can modify benzene toxicity [McHale et al. 2012], and exposure to ethanol reduces methyl ethyl ketone metabolism [Liira et al. 1990].

validation. This involves analysis of five replicates at three concentration levels. The recovery should be reproducible (better than 20% as measured by CV or RSD) for each of the concentration levels.

9) Robustness/ruggedness

Robustness or ruggedness is a measure of the susceptibility of a method to small changes that might occur during routine analysis. Validation of a method need not necessarily include ruggedness testing, but it can be very helpful during the method development phase. Problems that may occur during validation are often detected in initial ruggedness testing. Warnings can be included in the methodology about parameters that must be tightly controlled to achieve the desired levels of precision and accuracy.

While not every parameter of every type of method can be listed here, it is important for the analyst to consider every step of the method and strive to include ruggedness testing in each step. Some typical examples of variations include pH (of samples, mobile phases, extraction solvents), mobile phase composition, and columns (GC, LC, and extraction columns/cartridges with different lots or suppliers). Other sources can include variations in temperature (room, oven, column, processing, thawing, autosampler, etc.), flow rate (both in extraction and analysis), and various volumes and times used during the entire bioanalytical method.

10) Limit of detection and limit of quantification

The limit of detection (LOD), also referred to as the method detection limit (MDL), is the lowest concentration of an analyte that the bioanalytical procedure can reliably differentiate from background noise [FDA 2018]. The LOD should not be used for quantitative measurement but may be useful for semi-quantitative or qualitative determinations [Tiwari and Tiwari 2010]. Several literature methods for determining the LOD can be utilized [Bader et al. 2012; NIOSH 1995]. In brief, one method involves the analysis of five or more low-level standards (prepared in the appropriate matrix) that range from less than the expected LOD to no greater than 10 times the expected LOD. These standard responses are plotted and the regression equation and standard error (sy) are calculated. The LOD is then 3 sy/slope. Alternatively, analyze at least 10 standards near the anticipated LOD and calculate the standard deviation [ASTM 2020b]. The LOD (MDL) is reported as 3 times the standard deviation of the blank signal (correcting for background). The lower limit of quantitation (LLOQ) is concomitantly taken as 10 times the standard deviation of the blanks.

The LLOQ is the lowest concentration of analyte in a sample that can be quantified reliably with acceptable accuracy and precision. Calibration standards should include the lower and upper limits of quantitation (L/U LOQ) as values should not be extrapolated beyond the range of the calibration curve. The accuracy and precision criteria at each end of the curve have been found to be acceptable within the defined criteria. In addition, the analyte signal of the LLOQ should be at least 10 times sy/slope (or 3.33×LOD).

The LOD, LLOQ, and ULOQ should be reported in the validation report. These should also be reported when samples are analyzed. When reporting biomonitoring results, report results below the LOD as "not detected (ND)," and report results between the LOD and LLOQ numerically and enclose in parentheses to denote the greater imprecision of these results. It is also common to use one less significant figure when reporting results in this area.

11) Acceptance criteria for an analytical run

An analytical run consists of the following components:

- A matrix blank (a processed matrix sample without analyte and without internal standard)
- A zero sample (a processed matrix sample with internal standard)
- A minimum of six nonzero calibration levels
- A minimum of three levels of quality control (QC) samples in duplicate or a minimum of 5% of the total number of unknown samples, whichever is higher. Add QC samples in multiples of two when additional samples are needed
- Field/study/unknown samples

All samples should be processed as a single batch. Calibration standards and QC samples should be spiked independently using separately prepared stock solutions. A single batch is comprised of samples handled at the same time, by the same analyst, under homogeneous conditions.

The standard curve samples, blanks, QCs, and unknowns can be arranged as considered appropriate within the run and to support detection of drift or carryover over the course of the run. Acceptance criteria should be pre-established in the protocol, in the study plan, or in a standard operating procedure. In cases where a whole run consists of more than one batch, acceptance criteria should be applied to the whole run and to the individual batches.

12) Accuracy acceptance criteria for QC samples [EMA 2011; FDA 2018]

The accuracy values of the QC samples should be within ±15% for the upper concentration (50%–100% ULOQ) and within ±20% for the lower concentration (100%–200% LLOQ). At least 67% of the QC samples should be within these limits for a run to be accepted. Furthermore, at least 50% of the QC samples at each concentration should comply with this criterion.

In the case where the criteria are not fulfilled, the analytical run should be rejected. The samples can be re-extracted and analyzed, corrective action can be taken on the instrumentation, or other cause(s) of the failure investigated, analyzed, and corrected. In the case of the simultaneous determination of several analytes, there will be a calibration curve for each analyte in the method. If an analytical run is acceptable for one analyte but is rejected for another analyte, the data for the accepted analyte can be used.

13) Accuracy acceptance criteria for calibration standards [EMA 2011; FDA 2018]

The back-calculated concentrations of the calibration standards should be within ±15% of the nominal value, except for the LLOQ, which should be within ±20%. A minimum of 75% of the calibration standards should be within these limits for the analytical run to qualify. If one of the calibration standards does not meet these criteria, the standard should be discarded, provided the calibration model does not change, and the calibration curve without this standard should be re-evaluated and regression analysis performed.

If the discarded calibration standard is the LLOQ or the ULOQ, then the next calibration standard becomes the LLOQ or ULOQ, and the calibration range is narrower for that run. The revised calibration range should cover all concentrations of QC samples.

8 Conclusions

This chapter describes biomonitoring as a valuable tool for conducting research and public health surveillance, evaluating intervention effectiveness, and improving risk assessments. A major aim of biomarker research is to develop and validate biomarkers that reflect specific exposures or are quantitatively linked to adverse outcomes in humans to enable their use in risk prediction. The NRC identified several strategies to use biomarkers of effect to extrapolate dose and to evaluate dose response [NRC 2007]. Biomonitoring can be used not only to identify exposures but also to evaluate exposure trends over time. While several international agencies have developed biological exposure levels for some chemicals, OSHA has only mandated three tests for occupational compliance.

Some general considerations for biomonitoring research have been noted, such as the goals of biomonitoring for the study or program being initiated. The biological matrices of interest, whether blood, urine, exhaled breath, or some other specimen, will have an integral role in the selection of the biomarker most pertinent for the study. If the goal is to understand whether exposure occurred or not, it may be possible to measure the chemical or its metabolite in exhaled breath or urine. If the goal is to determine a health risk, then measurement of a biomarker of effect may be more appropriate.

Appropriate study design and statistical analyses in occupational research studies will aid in ensuring that studies of biomarkers have scientific rigor and will minimize uncertainty. Written standard operating procedures and a quality assurance program will help improve data quality and provide interpretable results. Adherence to field and laboratory safety procedures are imperative to protect staff from exposure to bloodborne pathogens. Ethical considerations should be at the forefront of any biomonitoring investigation.

Biomarkers need to be validated in the laboratory and for the population for which they will be used. The steps for laboratory validation of the analytical methods are covered in this chapter. Equally important is the population validation of biomonitoring methods, which is not in the scope of this chapter [Schulte and DeBord 2000]. Population validation determines the utility of the method in various population groups. It entails understanding interpersonal variability according to demographic and behavioral characteristics, determining the underlying prevalence of the marker, and identifying the optimal handling and logistical considerations [Schulte and DeBord 2000; Schulte and Perera 1997].

The potential of biomonitoring in occupational health is tempered by limitations in study design, interpretation of results, communication of results, and ethical issues. The ability of advances in existing and emerging technologies to develop new biomarker methods exceeds our practical ability to evaluate and validate all of them. The real challenges for occupational health professionals are to decide which measurement methods may be of value to understand what information biomarker measurements are providing, and, finally, to determine appropriate actions based on that information.

Disclaimer

Mention of any company or product does not constitute endorsement by the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention (CDC). In addition, citations to websites external to NIOSH do not constitute NIOSH endorsement of the sponsoring organizations or their programs or products. Furthermore, NIOSH is not responsible for the content of these websites. All web addresses referenced in this document were accessible as of the publication date.

9 References

ACGIH [2022]. TLVs and BEIs based on the documentation of the Threshold Limit Values for chemical substances and physical agents and Biological Exposure Indices. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, https://www.acgih.org/tlv-bei-guidelines/policies-procedures-presentations/overview.

Andersen ME, Clewell HJ III, Gargas ML, MacNaughton MG, Reitz RH, Nolan RJ, McKenna MJ [1991]. Physiologically based pharmacokinetic modeling with dichloromethane, its metabolite, carbon monoxide, and blood carboxyhemoglobin in rats and humans. Toxicol Appl Pharmacol *108*(1):14–27.

Andreasson U, Perret-Liaudet A, van Waalwijk van Doorn LJ, Blennow K, Chiasserini D, Engelborghs S, Fladby T, Genc S, Kruse N, Kuiperij HB, Kulic L, Lewczuk P, Mollenhauer B, Mroczko B, Parnetti L, Vanmechelen E, Verbeek MM, Winblad B , Zetterberg H, Koel-Simmelink M, Teunissen CE [2015]. A practical guide to immunoassay method validation. Front Neurol *6*:179, https://doi.org/10.3389%2Ffneur.2015.00179.

Angerer J, Aylward LL, Hays SM, Heinzow B, Wilhelm M [2011]. Human biomonitoring assessment values: approaches and data requirements. Int J Hyg Environ Health *214*(5):348–360, https://doi.org/10.1016/j.ijheh.2011.06.002.

Apel P, Angerer J, Wilhelm M, Kolossa-Gehring M [2017]. New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German Human Biomonitoring Commission. Int J Hyg Environ Health *220*(2, Pt A):152–166, https://doi.org/10.1016/j.ijheh.2011.06.002.

ANSES [2019]. Biological limit values for chemicals used in the workplace. Maisons-Alfort: French Agency for Food, Environmental and Occupational Health and Safety, https://www.anses.fr/en/content/biological-limit-values-chemicals-used-workplace.

Ashley K, Brisson MJ, White KT [2011]. Review of standards for surface and dermal sampling. J ASTM Int *8*(6):9, http://doi.org/10.1520/JAI103678.

ASTM [2020a]. ASTM D1356-20 Standard terminology relating to sampling and analysis of atmospheres. ASTM International: West Conshohocken, PA: American Society for Testing and Materials.

ASTM [2020b]. ASTM D6785-20 Determination of lead in workplace air using flame or graphite furnace atomic absorption spectrometry. ASTM International: West Conshohocken, PA: American Society for Testing and Materials.

ASTM [2022]. ASTM E691-22 Standard practice for conducting an interlaboratory study to determine the precision of a test method. ASTM International: West Conshohocken, PA: American Society for Testing and Materials.

Aylward LL, Hays SM [2011]. Biomonitoring-based risk assessment for hexabromocyclododecane (HBCD). Int J Hyg Environ Health *214*(3):179–187, https://doi.org/10.1016/j.ijheh.2011.02.002.

Aylward LL, Hays SM, Smolders R, Koch HM, Cocker J, Jones K, Warren N , Levy L, Bevan R [2014]. Sources of variability in biomarker concentrations. J Toxicol Environ Health B *17*(1):45–61, https://doi.org/10.1080/10937404.2013.864250.

Aylward LL, Kirman CR, Adgate JL, McKenzie LM, Hays SM [2012]. Interpreting variability in population biomonitoring data: role of elimination kinetics. J Expo Sci Environ Epidemiol *22*(4):398–404, http://doi.org/10.1038/jes.2012.35.

Aylward LL, Kirman CR, Schoeny R, Portier CJ, Hays SM [2013]. Evaluation of biomonitoring data from the CDC National Exposure Report in a risk assessment context: perspectives across chemicals. Environ Health Perspect *121*(3):287–294, https://doi.org/10.1289/ehp.1205740.

Bader M, Barr D, Göen T, Schaller KH, Scherer G, Angerer J [2012]. Reliability criteria for analytical methods. In: The MAK-collection for occupational health and safety Part IV: biomonitoring methods (2010). Vol. 12. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, https://doi.org/10.1002/3527600418.bireliabe0012.

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL [2005]. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect *113*(2):192–200, https://doi.org/10.1289/ehp.7337.

B'Hymer C, Krieg E Jr., Cheever KL, Toennis CA, Clark JC, Kesner JS, Gibson RL, Butler MA [2012]. Evaluation and comparison of urinary metabolic biomarkers of exposure for the jet fuel JP-8. J Toxicol Environ Health *75*(11):661–672, https://doi.org/10.1080/15287394.2012.688483.

Boeniger MF, Lowry LK, Rosenberg J [1993]. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. Am Ind Hyg Assoc J *54*(10):615–627, https://doi.org/10.1080/15298669391355134.

Brown JF Jr., Lawton RW [1984]. Polychlorinated biphenyl (PCB) partitioning between adipose tissue and serum. Bull Environ Contam Toxicol *33*:277–280, https://doi.org/10.1007/bf01625543.

Brown PO, Botstein D [1999]. Exploring the new world of the genome with DNA microarrays. Nat Genet *21*(1):33–37, https://doi.org/10.1038/4462.

Calafat AM, Longnecker MP, Koch HP, Swan SH, Hauser R, Goldman LR, Lanphear BP, Rudel RA, Engel SM, Teitelbaum SL, Whyatt RM, Wolff MS [2015]. Optimal exposure biomarkers for nonpersistent chemicals in environmental epidemiology. Environ Health Perspect *123*(7):A166–A168, https://doi.org/10.1289/ehp.1510041.

Carrieri M, Tevisan A, Bartolucci GB [2001]. Adjustment to concentration-dilution of spot urine samples: correlation between specific gravity and creatinine. Int Arch Occup Environ Health *74*(1):63–67, https://doi.org/10.1007/s004200000190.

Causon R [1997]. Validation of chromatographic methods in biomedical analysis. Viewpoint and discussion. J Chromatogr B Biomed Sci Appl *689*(1):175–180, https://doi.org/10.1016/s0378-4347(96)00297-6.

Cavallari JM, Osborn LV, Snawder JE, Kriech AJ, Olsen LD, Herrick RF, McClean MD [2012a]. Predictors of airborne exposures to polycyclic aromatic compounds and total organic matter among hot-mix asphalt paving workers and influence of work conditions and practices. Ann Occup Hyg *56*(2):138–147, https://doi.org/10.1093/annhyg/mer088.

Cavallari JM, Osborn LV, Snawder JE, Kriech AJ, Olsen LD, Herrick RF, McClean MD [2012b]. Predictors of dermal exposures to polycyclic aromatic compounds among hot-mix asphalt paving workers. Ann Occup Hyg *56*(2):125–137, https://doi.org/10.1093/annhyg/mer108.

CDC [2020]. Biosafety in microbiological and biomedical laboratories, 6th ed., Meechan PJ, Potts J, eds. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health, https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf.

CDC [2021]. National biomonitoring program. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, http://www.cdc.gov/biomonitoring/about.html.

CDC [2022]. National report on human exposure to environmental chemicals. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, https://www.cdc.gov/exposurereport/index.html.

Clewell HJ, Tan YM, Campbell JL, Andersen ME [2008]. Quantitative interpretation of human biomonitoring data. Toxicol Appl Pharmacol *231*(1):122–133, https://doi.org/10.1016/j.taap.2008.04.021.

Cone EJ, Caplan YH, Moser F, Robert T, Shelby MK, Black DL [2009]. Normalization of urinary drug concentrations with specific gravity and creatinine. J Anal Toxicol *33*(1):1–7, https://doi.org/10.1093/jat/33.1.1.

DeBord DG, Burgoon L, Edwards SW, Haber LT, Kanitz MH, Kuempel E, Thomas RS, Yucesoy B [2015]. Systems biology and early effects dose-response for OEL setting. J Occup Environ Hyg *12*(Suppl 1):S41–S54, https://doi.org/10.1080/15459624.2015.1060324.

Decker JA, DeBord DG, Bernard B, Dotson GS, Halpin J, Hines CJ, Kiefer M, Myers K, Page E, Schulte P, Snawder J [2013]. Recommendations for biomonitoring of emergency responders: focus on occupational health investigations and occupational health research. Mil Med *178*(1):68–75, https://doi.org/10.7205/milmed-d-12-00173.

Demchuk E, Yucesoy B, Johnson VJ, Andrew M, Weston A, Germolec D, De Rosa CT, Luster M [2007]. A statistical model for assessing genetic susceptibility as a risk factor in multifactorial diseases: lessons from occupational asthma. Environ Health Perspect *115*(2):231–234, https://doi.org/10.1289/ehp.8870.

DFG [2015]. List of MAK and BAT values 2015. Report 51. Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Bonn, Germany: Deutsche Forschungsgemeinschaft (DFG), https://onlinelibrary.wiley.com/doi/pdf/10.1002/9783527695539.oth1.

Droz PO [1989]. Biological monitoring I: sources of variability in human response to chemical exposure. Appl Ind Hyg *4*(1):F20–F24, https://doi.org/10.1080/08828032.1989.10389872.

EMA [2011]. Guideline on bioanalytical method validation. Committee for Medicinal Products for Human Use (CHMP). London: European Medicines Agency, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf.

Exley K, Aerts D, Biot P, Casteleyn L, Kolossa-Gehring M, Schwedler G, Castaño A, Angerer J, Koch HM, Esteban M, Schindler BK, Schoeters G, Hond ED, Horvat M, Bloemen L, Knudsen LF, Joas R, Joas A, Sepai O [2015]. Pilot study testing a European human biomonitoring framework for biomarkers of chemical exposure in children and their mothers: experiences in the UK. Environ Sci Pollut Res Int *22*(20):15821–15834, https://doi.org/10.1007/s11356-015-4772-4.

FDA [2018]. Bioanalytical method validation: guidance for industry. Silver Spring, MD: U.S. Department of Health and Human Services, Food and Drug Administration, https://www.fda.gov/media/70858/download.

Fiserova-Bergerova V [1993]. Biological monitoring VIII: interference of alcoholic beverage consumption with biological monitoring of occupational exposure of industrial chemicals. Appl Occup Environ Hyg *8*(9):757–760, https://doi.org/10.1080/1047322X.1993.10388193.

Gaines LGT, Fent KW, Flack SL, Thomasen JM, Ball LM, Zhou H, Whittaker SG, Nylander-French LA [2010]. Effect of creatinine and specific gravity normalization on urinary biomarker 1, 6-hexamethylene diamine. J Environ Monit *12*(3):591–599, https://doi.org/10.1039/b921073c.

Goldberger BA, Loewenthal B, Darwin WD, Cone EJ [1995]. Intrasubject variation of creatinine and specific gravity measurements in consecutive urine specimens of heroin users. Clin Chem *41*(1):116–117.

Gosho M, Nagashima K, Sato Y [2012]. Study designs and statistical analyses for biomarker research. Sensors *12*(7):8966–8986, https://doi.org/10.3390/s120708966.

GPO [2021]. Transportation – Hazardous materials regulation. Title 49 Subtitle B Chapter I Subchapter C Part 172. Government Publishing Office, https://www.ecfr.gov/current/title-49/subtitle-B/chapter-I/subchapter-C/part-172.

Green JM [1996]. A practical guide to analytical method validation. Anal Chem *68*(9):305A–309A, https://doi.org/10.1021/ac961912f.

Greenberg LA, Lester D [1947]. The metabolic fate of acetanilid and other aniline derivatives: the role of p-aminophenol in the production of methemoglobinemia after acetanilid. J Pharmacol Expo Ther *90*(2):150–153.

Hartmann C, Smeyers-Verbeke J, Massart DL, McDowall RD [1998]. Validation of bioanalytical chromatographic methods. J Pharm Biomed Anal *17*(2):193–218, https://doi.org/10.1016/s0731-7085(97)00198-2.

Hathaway GJ, Proctor NH, eds. [2004]. Proctor and Hughes' chemical hazards of the workplace, 5th ed. Hoboken, NJ: John Wiley & Sons, Inc.

HSE [2020]. Biological monitoring guidance values. In: EH40/2005 Workplace exposure limits, 4th edition. Liverpool, UK: Health and Safety Executive.

Hunter DJ [1997]. Methodologic issues in the use of biological markers in cancer epidemiology: cohort studies. In: Toniolo P, Boffetta P, Shuker DEG, Rothman N, Hulka B, Pearce N, eds. Application of biomarkers of cancer epidemiology. IARC Sci Publ (142):39–46.

Hunter DJ, Losina E, Guermazi A, Burstein D, Lassere MN, Kraus V [2010]. A pathway and approach to biomarker validation and qualification for osteoarthritis clinical trials. Curr Drug Targets *11*(5):536–545, https://doi.org/10.2174%2F138945010791011947.

IATA [2022]. Dangerous goods. Montreal, Canada: International Air Transport Association, http://www.iata.org/whatwedo/cargo/dgr/pages/index.aspx.

ICH [2005]. Validation of analytical procedures: text and methodology. Q2R1. International Conference on Harmonisation, https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf.

Irish National Accreditation Board [2019]. Guide to method validation for quantitative analysis in chemical testing laboratories (ISO 17025). PS15. Dublin, Ireland: Health and Safety Authority, https://www.inab.ie/inab-documents/mandatory-policy/guide-to-method-validation-for-quantitative-analysis-in-chemical-testing-laboratories-iso-17025-.pdf.

JSOH [2016]. Recommendation of occupational exposure limits (2016–2017). Japan Society for Occupational Health. J Occup Health *58*(5):489–518, https://doi.org/10.1539%2Fjoh.ROEL2016.

Landi MT, Caporaso N [1997]. Sample collection, processing and storage. In: Toniolo P, Boffetta P, Shuker DEG, Rothman N, Hulka B, Pearce N, eds. Application of biomarkers of cancer epidemiology. IARC Sci Publ (142):223–236

Lauwerys RR, Hoet P [1993]. Industrial chemical exposure: guidelines for biological monitoring. Ch. 1. Ann Arbor, MI: Lewis Publishers.

Levine L, Fahy JP [1945]. Evaluation of urinary lead determinations. J Ind Hyg Toxicol *27*:217–223.

Liira J, Riihimaki V, Engstrom K [1990]. Effects of ethanol on the kinetics of inhaled methyl ethyl ketone in man. Br J Ind Med *47*(5):325–330, https://doi.org/10.1136/oem.47.5.325.

Linder W, Wainer IW [1998]. Requirements for initial assay validation and publication in J. Chromatography B. Editorial. J Chromatogr B Biomed Sci Appl *707*(1–2):1–2, https://pubmed.ncbi.nlm.nih.gov/9613926/.

Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN [2003]. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet *33*(2):177–182, https://doi.org/10.1038/ng1071.

Manh HD, Kido T, Okamoto R, Xianliang S, Anh LT, Supratman S, Maruzeni S, Nishijo M, Nakagawa H, Honma S, Nakano T, Takasuga T, Nhu DD, Hung NN, Son LK [2014]. Serum dioxin levels in Vietnamese men more than 40 years after herbicide spraying. Environ Sci Technol *48*(6):3496–3503, https://doi.org/10.1021/es404853h.

McClean MD, Osborn LV, Snawder JE, Olsen LD, Kriech AJ, Sjodin A, Li Z, Smith JP, Sammons DL, Herrick RF, Cavallari JM [2012]. Using urinary biomarkers of polycyclic aromatic compound exposure to guide exposure-reduction strategies among asphalt paving workers. Ann Occup Hyg *56*(9):1013–1024, https://doi.org/10.1093/annhyg/mes058.

McHale CM, Zhang L, Smith MT [2012]. Current understanding of the mechanism of benzene-induced leukemia in humans: implications for risk assessment. Carcinogenesis *33*(2):240–252, https://doi.org/10.1093/carcin/bgr297.

Menke A, Muntner P, Silbergeld EK, Platz EA, Guallar E [2009]. Cadmium levels in urine and mortality among U.S. adults. Environ Health Perspect *117*(2):190–196, https://doi.org/10.1289/ehp.11236.

Mikkelsen SR, Cortón E [2004]. Validation of new bioanalytical methods. In: Bioanalytical chemistry. Hoboken, NJ: John Wiley & Sons, Inc., https://doi.org/10.1002/0471623628.ch16.

Miller RC, Brindle E, Holman DJ, Shofer J, Klein NA, Soules MR, O'Connor KA [2004]. Comparison of specific gravity and creatinine for normalizing urinary reproductive hormone concentrations. Clin Chem *50*(5):924–932, https://doi.org/10.1373/clinchem.2004.032292.

Needham LL, Ozkaynak H, Whyatt RM, Barr DB, Wang RY, Naeher L, Akland G, Bahadori T, Bradman A, Fortmann R, Liu LJS, Morandi M, O'Rourke MK, Thomas K, Quackenboss J, Ryan PB, Zartarian V [2005]. Exposure assessment in the national Children's Study: introduction. Environ Health Perspect *113*(8):1076–1082, https://doi.org/10.1289/ehp.7613.

NIOSH [1995]. Guidelines for air sampling and analytical method development and evaluation. By Kennedy ER, Fischbach TJ, Song R, Eller PM, Shulman SA. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 95-117, https://www.cdc.gov/niosh/docs/95-117/default.html.

NIOSH [1998]. Application of biological monitoring methods: Chapter F. By Teass AW, Biagini RE, DeBord DG, Hull RD. In: Eller PM, Cassinelli ME, O'Connor PF, eds. NIOSH manual of analytical methods. 4th ed. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 98-119, https://www.cdc.gov/niosh/docs/2003-154/pdfs/chapter-f.pdf.

NIOSH [2010]. Genetics in the workplace: implications for occupational safety and health. By DeBord DG, Schulte PA, Butler MA, McCanlies E, Reutman S, Ruder A, Schill A, Schubauer-Berigan M, Schuler C, Weston A. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2010-101, https://www.cdc.gov/niosh/docs/2010-101/default.html.

NIOSH [2012]. Medical surveillance for health care workers exposed to hazardous drugs. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2013-103, http://www.cdc.gov/niosh/docs/wp-solutions/2013-103/pdfs/2013-103.pdf.

NIOSH [2021]. Adult blood lead epidemiology and surveillance (ABLES). Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention,

National Institute for Occupational Safety and Health, http://www.cdc.gov/niosh/topics/ables/default.html.

NRC [1987]. Biological markers in environmental health research. Committee on Biological Markers of the National Research Council. Environ Health Perspect *74*:3–9, https://doi.org/10.1289/ehp.74-1474499.

NRC [2006]. Human biomonitoring for environmental chemicals. National Research Council, Washington, DC: The National Academies Press.

NRC [2007]. Toxicity testing in the 21st century: a vision and a strategy. National Research Council, Washington, DC: The National Academies Press.

Ogata M, Fiserova-Bergerova V, Droz PO [1993]. Biological monitoring VII: occupational exposures to mixture of industrial chemicals. Appl Occup Environ Hyg *8*(7):609–617, https://doi.org/10.1080/1047322x.1993.10388168.

OSHA [no date]. Bloodborne pathogens and needlestick prevention. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration, https://www.osha.gov/SLTC/bloodbornepathogens/index.html. Date accessed: July 21, 2022.

OSHA [1978]. Lead. Occupational Health and Safety Standard 1910.1025. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration, https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10030.

OSHA [1980]. Benzene. Occupational Health and Safety Standard 1910.1028. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration, https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10042.

OSHA [1981]. Cadmium. Occupational Health and Safety Standard 1910.1027. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration, https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10035.

OSHA [1992]. Bloodborne pathogens. Occupational Health and Safety Standard 1910.1030. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration, https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030.

Peters FT, Maurer HH [2015]. Review: bioanalytical method validation—How, how much and why? https://orion.gtfch.org/cms/images/stories/media/tk/tk68_3/Peters.pdf.

Richards DM, Kraus JF, Kurtz P, Borhani NO, Mull R, Winterlin W, Kilgore WW [1978]. A controlled field trial of physiological responses to organophosphate residues in farm workers. J Environ Pathol Toxicol *2*(2):493–512.

Rosenberg J [1994]. Biological monitoring IX: concomitant exposure to medications and industrial chemicals. Appl Occup Environ Hyg *9*(5):341–345, https://doi.org/10.1080/1047322X.1994.10388324.

Rosenberg J, Fiserova-Bergerova V, Lowry LK [1989]. Biological monitoring IV: measurements in urine. Appl Ind Hyg *4*(4):F16–F21, https://doi.org/10.1080/08828032.1989.10390349.

Sauve J, Levesque M, Huard M, Drolet D, Lavoue J, Tardif R, Truchon G [2015]. Creatinine and specific gravity normalization in biological monitoring of occupational exposures. J Occup Environ Hyg *12*(2):123–129, https://doi.org/10.1080/15459624.2014.955179.

Schulte PA [2005]. The use of biomarkers in surveillance, medical screening, and intervention. Mutat Res, Fundam Mol Mech Mutagen *592*(1–2):155–63, https://doi.org/10.1016/j.mrfmmm.2005.06.019.

Schulte PA, DeBord DG [2000]. Public health assessment of genetic information in the occupational setting. In: Khoury M, Burke W, Thomson E, eds. Genetics and public health in the 21st century. New York: Oxford Press, https://doi.org/10.1093/acprof:oso/9780195128307.001.0001.

Schulte PA, Hauser JE [2012]. The use of biomarkers in occupational health research, practice, and policy. Toxicol Lett *213*(1):91–99, https://doi.org/10.1016/j.toxlet.2011.03.027.

Schulte PA, Perera FP [1993]. Validation. In: Schulte PA, Perera FP, eds. Molecular epidemiology: principles and practices. San Diego, CA: Academic Press.

Schulte PA, Perera FP [1997]. Transitional studies. In: Toniolo P, Boffetta P, Shuker DEG, Rothman N, Hulka B, Pearce N, eds. Application of biomarkers of cancer epidemiology. IARC Sci Publ (142):19–29.

Schulte PA, Whittaker C, Curran CP [2015]. Considerations for using genetic and epigenetic information in occupational health risk assessment

and standard setting. J Occup Environ Hyg *12*(Suppl 1):S69–S81, https://doi.org/10.1080%2F15459624.2015.1060323.

Schulz C, Wilhelm M, Heudorf U, Kolossa-Gehring M [2011]. Update of the reference and HBM values derived by the German Human Biomonitoring Commission. Int J Hyg Environ Health *215*(1):26–35, https://doi.org/10.1016/j.ijheh.2011.06.007.

Scinicariello F, Yesupriya A, Chang MH, Fowler B [2010]. Modification by ALAD of the association between blood lead and blood pressure in the U.S. population: results from the third National Health and Nutrition Examination Survey. Environ Health Perspect *118*(2):259–264, https://doi.org/10.1289/ehp.0900866.

SCOEL [2014]. List of recommended health-based biological limit values (BLVs) and biological guidance values (BGVs), European Scientific Committee on Occupational Exposure Limits, http://ec.europa.eu/social/main.jsp?catId=148&intPageId=684&langId=en.

Sexton K, Needham LL, Pirkle JL [2004]. Human biomonitoring of environmental chemicals: measuring chemicals in human tissues is the "gold standard" for assessing the people's exposure to pollution. Am Sci *92*(1):38–45, http://www.jstor.org/stable/27858331.

Smeraglia J, Baldrey SF, Watson D [2002]. Matrix effects and selectivity issues in LC-MS-MS. Chromatographia *55*:S95–S99, http://doi.org/10.1007/BF02493363.

Spencer K [1986]. Analytical reviews in clinical biochemistry: the estimation of creatinine. Ann Clin Biochem *23*(Part 1):1–25, https://doi.org/10.1177/000456328602300101.

St-Amand A, Werry K, Aylward LL, Hayes SM, Nong A [2014]. Screening of population level biomonitoring data from the Canadian Health Measures Survey in a risk-based context. Toxicol Lett *231*(2):126–134, https://doi.org/10.1016/j.toxlet.2014.10.019.

SUVA [2019]. Limit values at the workplace. MAK/BAT values (explanations) physical influences, physical loads. Swiss Accident Insurance Fund (SUVA) , https://www.suva.ch/de-CH/material/Richtlinien-Gesetzestexte/erlaeuterungen-zu-den-grenzwerten, [in German].

Taylor L, Jones RL, Ashley K, Deddens JA, Kwan L [2004]. Comparison of capillary earlobe and venous blood monitoring for occupational lead surveillance. J Lab Clin Med *143*(4):217–224, https://doi.org/10.1016/j.lab.2003.12.011.

Theodorsson E [2012]. Validation and verification of measurement methods in clinical chemistry. Bioanalysis *4*(3):305–320, https://doi.org/10.4155/bio.11.311.

Thomas RS, Clewell HJ III, Allen BC, Wesselkamper SC, Wang NCY, Lambert JC, Hess-Wilson JK, Zhao QJ, Andersen ME [2011]. Application of transcriptional benchmark dose values in quantitative cancer and noncancer risk assessment. Toxicol Sci *120*(1):194–205, https://doi.org/10.1093/toxsci/kfq355.

Tiwari G, Tiwari R [2010]. Bioanalytical method validation: an updated review. Pharm Methods *1*(1):25–38, https://doi.org/10.4103%2F2229-4708.72226.

Trufelli H, Palma P, Famiglini G, Cappiello A [2011]. An overview of matrix effects in liquid chromatography-mass spectrometry. Mass Spectrom Rev *30*(3):491–509, https://doi.org/10.1002/mas.20298.

UNODC [2009]. Guidance for the validation of analytical methodology and calibration of equipment used for testing of illicit drugs in seized materials and biological specimens. Vienna, Austria: United Nations Office on Drugs and Crime, http://www.unodc.org/documents/scientific/validation_E.pdf.

Valcke M, Haddad S [2015]. Assessing human variability in kinetics for exposures to multiple environmental chemicals: a physiologically based pharmacokinetic modeling case study with dichloromethane, benzene, toluene, ethylbenzene, and m-xylene. J Toxicol Environ Health A *78*(7):409–431, https://doi.org/10.1080/15287394.2014.971477.

Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, Shah VP, Skelly JP, Swann PG, Weiner R [2007]. Workshop/conference report: Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. AAPS J *9*(1):E30–E42, http://doi.org/10.1208/aapsj0901004.

WADA [2016]. Endogenous anabolic androgenic steroids. Measurement and reporting. World Anti-Doping Agency Technical Document (WADA) TD2016EAAS:1–16, https://www.wada-ama.org/sites/default/files/resources/files/wada-td2016eaas-eaas-measurement-and-reporting-en.pdf.

WHO [2001]. Environmental Health Criteria 222. Biomarkers in risk assessment: validity and validation. Geneva, Switzerland: World Health Organization, https://inchem.org/documents/ehc/ehc/ehc222.htm.

Wieling J, Hendriks G, Tamminga WJ, Hempenius J, Mensink CK, Oosterhuis B, Jonkman JHG [1996]. Rational experimental design for bioanalytical methods validation. Illustration using an assay method for total captopril in plasma. J Chromatogr A *730*(1–2):381–394, https://doi.org/10.1016/0021-9673(96)00006-4.

Yeh H-C, Lin Y-S, Kuo C-C, Weidemann D, Weaver V, Fadrowski J, Neu A, Navas-Acien A [2015]. Urine osmolality in the US population: implications for environmental biomonitoring. Environ Res *136*:482–490, https://doi.org/10.1016%2Fj.envres.2014.09.009.

Zidek A, Macey K, MacKinnon L, Patel M, Poddalgoda D, Zhang Y [2017]. A review of human biomonitoring data used in regulatory risk assessment under Canada's Chemicals Management Program. Int J Hyg Environ Health *220*(2):167–178, https://doi.org/10.1016/j.ijheh.2016.10.007.

Zong G, Grandjean P, Wu H, Sun Q [2015]. Circulating persistent organic pollutants and body fat distribution: evidence from NHANES 1999–2004. Obesity (Silver Spring) *23*(9):1903–1910, https://doi.org/10.1002/oby.21161.