Review

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Using Biomonitoring Equivalents to interpret human biomonitoring data in a public health risk context

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ABSTRACT: Increasingly sensitive analytical tools allow measurement of trace concentrations of chemicals in human biological media in persons from the general population. Such data are being generated by biomonitoring programs conducted by the US Centers for Disease Control and other researchers. However, few screening tools are available for interpretation of such data in a health risk assessment context. This review describes the concept and implementation of Biomonitoring Equivalents (BEs), estimates of the concentration of a chemical or metabolite in a biological medium that is consistent with an existing exposure guidance value such as a tolerable daily intake or reference dose. The BE approach integrates available pharmacokinetic data to convert an existing exposure guidance value into an equivalent concentration in a biological medium. Key concepts regarding the derivation and communication of BE values resulting from an expert workshop held in 2007 are summarized. BE derivations for four case study chemicals (toluene, 2,4-dichlorophenoxyacetic acid, cadmium and acrylamide) are presented, and the interpretation of biomonitoring data for these chemicals is presented using the BE values. These case studies demonstrate that a range of pharmacokinetic data and approaches can be used to derive BE values; fully developed physiologically based pharmacokinetic models, while useful, are not required. The resulting screening level evaluation can be used to classify these compounds into relative categories of low, medium and high priority for risk assessment follow-up. Future challenges related to the derivation and use of BE values as tools in risk management are discussed. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: biomonitoring; screening; risk assessment; pharmacokinetics; Biomonitoring Equivalents

Introduction and Background

Biomonitoring — defined here as the measurement of parent chemical or metabolite in a biological sample — has long been used in the occupational realm as one tool for assessing and controlling exposure to chemicals in the workplace (Fiserova-Bergerova, 1987, 1990). However, over the past 10 years, advances in analytical sensitivity and an increasing focus on exposures to chemicals in the environment has led to more widespread use of biomonitoring as a tool for studies of general population groups. In many cases, biomonitoring for one or more specific chemicals has been incorporated as one aspect of exposure assessment in epidemiological studies of specific populations. However, the biomonitoring program underway at the US Centers for Disease Control and Prevention (CDC) and similar programs being developed by Health Canada, the state of California and being considered in Europe, differ from such studies in that the programs are not designed to test hypotheses among specific groups. Rather, these programs are designed to generate population-representative data on the presence and concentrations of a wide range of chemicals in human biological samples (usually blood or urine).

The growing availability of such data for hundreds of chemicals provides an opportunity, as well as challenges, for risk managers. Because biomonitoring data integrate exposures from multiple exposure pathways, the data provide valuable exposure data unavailable from any other source; such data have been referred to as a 'gold standard' for exposure assessment (Needham *et al*., 1999). However, because our current risk assessment paradigm has focused on external doses via specific exposure routes or external media concentrations, there is no direct method available for interpretation of the measured blood or urine concentrations of chemicals in a risk assessment context. It is not possible to directly compare an external dose, expressed as an intake in mg kg^{-1}-day to an internal concentration, generally expressed as mg l^{-1} or μg l^{-1}. With very few exceptions (for example, lead or mercury in blood), no human clinical screening criteria exist to allow assessments of biomonitoring data in a public health risk context, including the use of the current risk assessment framework. Development of such human clinical blood- or urine- level screening criteria such as those available for lead and mercury is resource-intensive and requires, by definition, extensive medical studies of effects in populations that have been exposed. Thus, similar human clinical blood- or urine-level criteria are unlikely to be available in the near future for most of the broad range of chemical substances now being analyzed and detected in biomonitoring programs.

The National Research Council (NRC) recognized that 'In spite of its [human biomonitoring] potential, tremendous challenges surround the use of biomonitoring, and our ability to generate biomonitoring data has exceeded our ability to interpret what the data mean to public health' (NRC, 2006). The NRC recognized that methods for placing human biomonitoring data in a health

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risk context were critical for biomonitoring to achieve its full potential and that a framework for the communication of this interpretation was equally important.

In recognition of the lack of screening tools for assessment of the broad range of chemicals included in the CDC (and other) Biomonitoring Programs, Hays *et al.* (2007) proposed the concept of Biomonitoring Equivalents (BEs). A BE is defined as the concentration of a chemical or metabolite in a biological medium that is consistent with an existing exposure guidance value such as a tolerable daily intake (TDI) or reference dose (RfD). These available chemical-specific exposure guidance values have been developed and used for the past 40 years by regulatory agencies to identify exposures to chemicals that are considered to be without likely adverse effects in the general population. For example, the US Environmental Protection Agency (USEPA) Reference Dose is a defined as 'an estimate of a daily oral exposure for a given duration to the human population including susceptible subgroups that is likely to be without an appreciable risk of adverse health effects over a lifetime' (http://www.epa.gov/IRIS/gloss8_arch.html). The BE concept incorporates available pharmacokinetic data (in either humans or in the species used in studies underlying the derivation of the exposure guidance value) to derive estimates of biomarker concentrations consistent with those exposure guidance values. Thus, BE values could be used as benchmarks to screen biomonitoring data sets to identify chemicals with measured values well below, near, at or above concentrations consistent with the existing exposure guidance values. This screening process could assist risk managers in using biomonitoring data to prioritize chemicals for additional evaluation or risk management actions.

BEs are similar in fundamental concept to the Biological Exposure Indices® (BEIs) and other similar biological monitoring tools used in the occupational realm (Fiserova-Bergerova, 1990). However, the application of this approach to environmental exposures and environmental exposure guidance values requires consideration of a number of additional factors. In recognition of the many technical and communications challenges likely to be encountered in the implementation of the BE concept, an expert workshop was convened in June 2007 to address a series of charge questions on the derivation and communication of BE values (Hays *et al*., 2008a). The workshop consisted of experts in pharmacokinetics, risk assessment, medical ethics and risk communication from a broad range of government, academic and industrial backgrounds. The workshop was funded by a range of sponsors including government agencies (Health Canada, USEPA) and industry trade groups. The discussions were informed by examples drawn from several case study chemicals: toluene, cadmium, acrylamide and 2,4-dichlorophenoxyacetic acid (2,4-D). The results of the workshop and the case studies are presented in detail in a series of papers (Aylward and Hays, 2008; Aylward *et al*., 2008; Hays and Aylward, 2008; Hays *et al*., 2008b,c; LaKind *et al*., 2008).

This review provides an overview of the key results of these workshop deliberations and examples of the application of the BE concept to interpretation of current biomonitoring data for the case study chemicals. The use of a variety of types of pharmacokinetic data, including, but not limited to, physiologically based pharmacokinetic (PBPK) models, is described. Finally, we present a discussion of continuing challenges and next steps in the development and application of the BE concept.

Key Concepts and Methods

The BE expert workshop addressed charge questions on both technical issues related to methods for the derivation of BE values and on issues related to the appropriate use and communication of BE values in the context of interpretation of biomonitoring data. A complete discussion of the workshop outcomes is presented in Hays *et al*. (2008b) and LaKind *et al*. (2008). The following overview focuses on key concepts and guidelines emerging from the workshop.

Derivation of BE Values

The original conception of the BE recognized the flexibility in approaches for translating an external dose to a corresponding BE (Fig. 1). If human pharmacokinetic information is available, a target external dose can be converted into the corresponding expected internal dose (concentration of parent compound or metabolite in blood and/or urine) in humans (pathway 1 in Fig. 1). Alternatively, if pharmacokinetic data are available in the animal species used in the study that provides the point of departure (POD) on which the exposure guidance value is based, the internal dose in the animal at the POD can be estimated and then the appropriate uncertainty factors (UFs) corresponding to those used in the derivation of the exposure guidance value can be

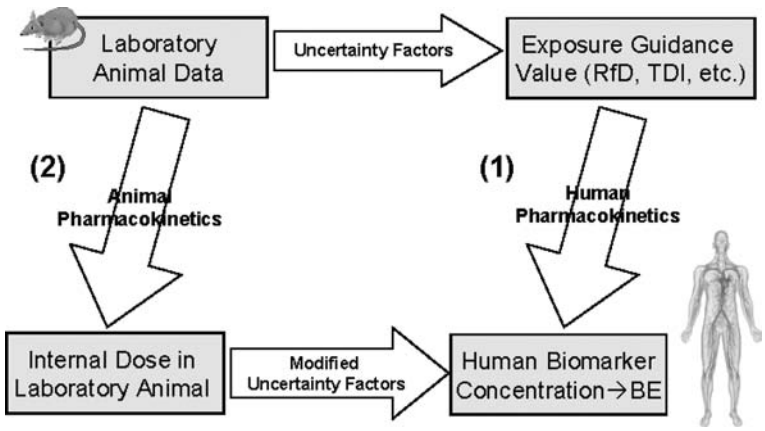

Figure 1. Parallelogram illustrating the BE concept. Pathways 1 and 2 illustrate the potential uses of human and animal pharmacokinetic data to identify BE values. Figure from Hays *et al*. (2008b).

applied to derive a BE (pathway 2, Fig. 1). This flexibility offers many advantages and affords the opportunity to leverage an internal dose based reconstruction of the exposure guidance value when sufficient information in known and available.

The most straightforward approach, but perhaps the least informative, involves pathway 1, but this requires some information on the pharmacokinetics of the compound(s) of interest in humans. The alternative approach, which involves reconstructing the exposure guidance value on an internal dose basis (pathway 2) may provide a more toxicologically relevant estimate of the biomarker concentration that is consistent with the exposure guidance value (Andersen, 1987, 1995; Andersen *et al*., 1995). This approach requires at least limited information on the mode of action for the associated toxic endpoint of interest to allow identification of the critical or at least a relevant internal dose metric. When sufficient information is available to follow the internal dose reconstruction pathway, uncertainty in the risk assessment may be reduced, and is informed by internal dose-based risk assessment concepts (Hays *et al*., 2008b).

The BE derivation process results in two estimates of the biomarker concentration at relevant points in the risk assessment process: the BE_{POD}, consistent with the human equivalent point of departure (after application of all adjustment factors to account for duration, lowest observed adverse effect level [LOAEL] to no-observed adverse effect level [NOAEL], and interspecies extrapolations), and the final BE value, consistent with the exposure guidance value (accounting for intraspecies and other uncertainty factors, such as database uncertainty factors). Uncertainty factor components applied on the derivation of the external exposure guidance value are retained, except under specific circumstances when inter- or intra-species extrapolations are made using a dose metric that is equal to or directly relevant to the critical dose metric. In this situation, pharmacokinetic components of the uncertainty factors may be replaced by the modeled or measured pharmacokinetic data. In this respect, the BE methodology is consistent in concept with the derivation and application of chemical-specific adjustment factors (Dorne and Renwick, 2005). Use of toxicologically relevant biomarkers as the metric for exposure measurement and setting benchmarks bypasses the need to model the pharmacokinetics that relate external to internal doses (either within or between species). Measured biomarker concentrations reflect these processes explicitly on a chemical-specific basis, replacing pharmacokinetic uncertainty factor components (either default or chemical-specific). Key steps in the BE derivation process for non-cancer and cancer endpoints are described in detail in Hays *et al*. (2008b), and outlined briefly below.

Identification of the Target Organ and any Available Understanding Regarding the Mode of Action for Toxicity. Evaluate the animal or human exposure-response data used as the basis for the derivation of the exposure guidance value to assess the understanding of mode of action. Based on that understanding, an assessment of the critical or relevant dose metrics should be made. Even though a specific mechanism of action may not be known, often information is available that will allow an assessment of whether the toxic moiety is the parent compound or a metabolite. Similarly, some information may be available regarding relevant dose metrics for the toxic moiety, for example, target organ average or area under the curve (AUC) exposure vs peak exposures.

Identification of Potential Biomarkers. Selection of biomarkers for a given chemical should consider several factors so that the analytical data can be interpreted in a health risk context. The biomarker should be specific to the chemical of interest. Ideally, the biomarker should be the toxic moiety, or, failing that, a marker just upstream in the metabolic pathway from the toxic moiety or a marker that is directly related to the toxic moiety. Another consideration is the stability of the biomarker. Longer-lived species provide a more stable assessment of exposure. Finally, the invasiveness of the required sampling is also a consideration. In many cases, not all of these criteria can be satisfied with a single biomarker. However, evaluation of potential biomarkers on these characteristics provides a useful framework to assist in recognition of the strengths and limitations of the resulting biomonitoring data.

Identify and Assess Available Pharmacokinetic Data and Models in Humans and the Relevant Animal Species. Available pharmacokinetic data, including measurements of blood or tissue concentrations of the parent or metabolite compound in the relevant species used in the exposure guideline derivation, should be identified and assessed for relevance in the BE derivation process. Although fully validated PBPK models are useful, they are not necessary; examples of the use of more limited pharmacokinetic data are presented below in the case studies. The derivation of a BE will involve estimating the relevant internal dose associated with the POD from the critical study used to derive the exposure guidance value. This can be derived using measured data (for example, measured serum concentrations of acrylamide and glycidamide in rats dosed at or near the point of departure for the risk assessment — see discussion below), simple pharmacokinetic approaches that involve linear relationships (see the acrylamide BE below) or a simple one-compartment pharmacokinetic model, or a PBPK model (see the toluene BE below). Likewise, extrapolations between the relevant internal dose metric and the measured biomarker may also be required (see the acrylamide BE below) involving similar types of methods of extrapolation.

Calculating BEs for Biomarkers in Urine

Derivation of BE values for urinary biomarkers may proceed along a parallel path, or may be derived based on a simple mass balance approach. Under the assumption of steady-state exposure (consistent with the definition of most chronic exposure guidance values), a mass balance may be assumed between the amount taken in and the amount of metabolite excreted. If key metabolites representing a substantial fraction of the parent compound are known, the urinary concentration of these metabolites consistent with the exposure guidance value can be estimated, taking into account typical daily urinary volumes (Perucca *et al*., 2007) and/or daily creatinine excretion rates (Mage *et al*., 2004). While these parameters vary due to hydration status or individual factors affecting creatinine excretion rates, the use of typical values can provide a central estimate of typical urinary concentration consistent with the exposure guidance value, appropriate for use as a screening value.

Confidence Rating

An important component of the BE derivation process is to make a judgment about the confidence of the BE. In particular, two components of the BE are assessed for confidence. The first is a determination of how relevant the biomarker is to the relevant internal dose metric (if known) should be made. The second is

an assessment of how robust the available pharmacokinetic data are for making the extrapolations necessary in calculating the BE. For each component, a low, medium or high measure should be given and communicated along with the BEs.

Discussion of Variability and Uncertainty

Each BE dossier also presents a discussion of factors that contribute to variability or uncertainty regarding the BE estimates. These factors include age, gender, smoking status or other lifestyle factors, genetic variability, uncertainty in pharmacokinetic data and models, and other factors as appropriate. Where possible, the magnitude of variability or uncertainty is described.

Communication and Interpretation using BE Values

An important focus of the BE expert workshop discussions was the challenge of appropriately communicating both the uses and limitations of BE values in the interpretation of biomonitoring data. BE values are designed to be used as screening tools to allow for initial evaluation of biomonitoring data in the context of existing risk assessments. Measured concentrations of the biomarker in population studies (such as those conducted by CDC through the NHANES program) can be compared with the available BE values to assess whether biomarker concentrations are well below, near or above the appropriate BE value. In this respect, the interpretation and uses of these values are consistent with the interpretation and uses of the underlying exposure guidance values. That is, the BE values can be used for prioritization when multiple chemicals are being assessed, in order to identify those that appear to be well below, near or above levels consistent with the underlying exposure guidance values. The resulting communication message is thus one of relative priority (low, medium or high) for further risk assessment follow-up. Biomarker concentration regions of low, medium and high priority are identified, respectively, as those below the BE, those between the BE and the BE_{POD}, and those above the BE_{POD}. Figure 2 presents a generic schematic for conveying the regions of relative priority for risk assessment follow-up based on the BE values.

Consistent with this framework for interpretation, several limitations and restrictions on the use of BE values are required. BE values are not diagnostic, and cannot be used to predict the likelihood of an adverse effect in individuals or a population; they are not bright lines separating 'safe' from 'unsafe' exposures. The exposure guidance values underlying the BE value derivations generally are framed in terms of lifetime average exposures; biomonitoring data generally present snapshots reflecting current exposures, so this needs to be taken into account when considering conclusions regarding lifetime risks based on the relationship between such data and the BE values. These limitations should be explicitly conveyed when BE values are used to interpret biomonitoring data.

Interpretation of data for highly transient (rapidly metabolized and eliminated compounds) is particularly challenging. Because both peaks and very low (or non-detectable) concentrations of biomarkers may occur under exposure conditions consistent with the exposure guidance values, BE values for such compounds are targeted at average concentrations, and interpretation of biomonitoring data for a population should likewise consist of a comparison of central tendency measures (rather than upper bound or extreme values) to the BE values. Extra caution should be used in drawing any conclusions regarding exposures for such compounds based on cross-sectional biomonitoring studies.

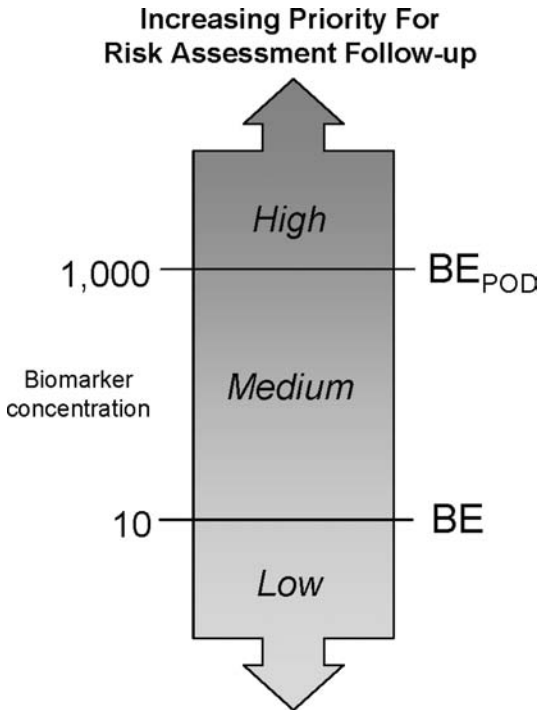

Figure 2. Generic figure for illustrating the relative ranges of biomarker concentrations associated with increasing levels of priority for risk assessment follow-up. The biomarker concentrations associated with the human-equivalent NOAEL point of departure are illustrated, as is the final BE value (the numerical designations in this figure are hypothetical for illustration purposes only).

Case Studies

Chemical-specific BEs have been developed and published for several chemicals including toluene (Aylward *et al*., 2008), 2,4-dichlorophenoxyacetic acid (2,4-D; Aylward and Hays, 2008), cadmium (Hays *et al*., 2008c) and acrylamide (Hays and Aylward 2008). Each dossier contains information on the pharmacokinetics, available biomarkers, exposure guidance values, methods for calculating BE values, BEs for each exposure guidance value and discussions of sources of uncertainty in calculating the BE and factors that would be expected to cause variability in biomonitoring levels. The case study chemicals were chosen to present a variety of challenges based on the type of pharmacokinetic data and models available and the toxicology underlying the exposure guidance value derivations. Following are brief overviews of the derivation of BE values for one specific exposure guidance value for each of the case study chemicals. These descriptions highlight the variety of approaches that can be taken in estimating BE values based on the individual chemical data sets. Additional details regarding the derivation of these BE values and the BE values consistent with other exposure guidance values for these chemicals can be found in the individual BE dossiers.

Toluene

Inhalation exposure guidance values for toluene are available from several agencies including the USEPA, Health Canada, the

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World Health Organization and the ATSDR. Each of these values is based on consideration of data from human occupational studies regarding potential neurotoxicity following chronic inhalation exposure to toluene. The following describes the use of the available human PBPK model to derive a BE value consistent with Health Canada's inhalation tolerable concentration (TC) (Health Canada, 1992).

The pharmacokinetics of toluene have been studied extensively in human volunteers and persons occupationally exposed as well as in laboratory animals. Toluene is well absorbed following inhalation and oral exposure, undergoes metabolism, principally via CYP2E1 and its metabolites are excreted in urine. Toluene is also eliminated as a parent compound in urine and exhaled air. Detailed physiologically based PBPK models for toluene in humans have been developed and can accurately predict blood levels associated with a variety of inhalation exposure regimens (Tardif *et al*., 1993, 1995; Jang, 1996; Pierce *et al*., 1996). The models are quite similar in structure and parameterization; for the purposes of the BE derivation, the model of Tardif *et al*. (1995), as parameterized and implemented by Nong *et al*. (2006) was used.

Several potential biomarkers are available for assessing internal exposure to toluene, including toluene in blood, urine and exhaled air, and various metabolites excreted in urine, including hippuric acid, *ortho*-cresol, *S*-*p*-toluylmercapturic acid and *S*-benzylmercapturic acid. Identification of relevant dose metrics depends upon the health endpoints that are the bases of the health-based screening values. Neurological responses following inhalation exposure to toluene in humans or oral exposure in rats and mice are likely to be related directly to brain concentrations of toluene (rather than metabolites), which in turn are directly related to blood concentrations (van Asperen *et al*., 2003; Benignus *et al*., 2007; Bushnell *et al*., 2007). However, there are insufficient data to conclusively identify whether peak or average toluene concentration in blood is the most appropriate dose metric for various neurological responses. The direct correlation between toluene blood concentration and neurological responses supports use of blood concentration of toluene as a biomarker, and under chronic exposure conditions, average blood concentration should be directly relevant.

The general approach for the derivation of BE values for the inhalation exposure guidelines is presented in Fig. 3 (Aylward *et al*., 2008, provide additional details). The Health Canada inhalation TC is derived based on human data. Thus, the derivation process does not involve an interspecies extrapolation. Briefly, the process is as follows:

- Calculate the BE_{POD}. The steady-state blood concentration in humans exposed at the duration- and LOAEL-to-NOAEL adjusted POD (based on human study data) was modeled using the PBPK model described above. Because blood toluene concentration has been identified as a directly relevant dose metric for neurological effects, the relevant internal dose metric and the monitored biomarker concentration are the same. The duration-adjusted NOAEL used as the basis of the Health Canada TC was 38 ppm in air. Using the PBPK model, the predicted steady-state toluene blood concentration associated with chronic exposure at this air concentration is approximately 135 $\mu g\ l^{-1}$. This modeled blood concentration is the BE_{POD} value.
- Calculate the BE via application of relevant intraspecies uncertainty factor(s) and any additional applicable uncertainty factors identified by the organizations that derived the oral exposure guidelines initially (for example, database uncertainty factors sometimes applied by USEPA). Because the measured biomarker is identical to the internal dose metric of interest, blood toluene concentration, direct measurement of this blood toluene concentration replaces application of the pharmacokinetic component of the intraspecies uncertainty factor in derivation of the BE values (Hays *et al*., 2008b); only the pharmacodynamic factor of $10^{0.5}$ is appropriate on an

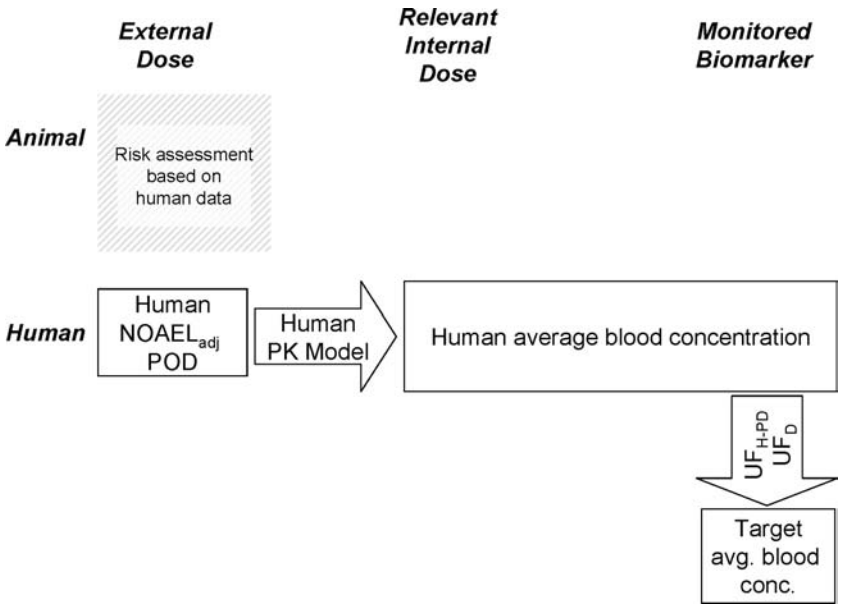

Figure 3. Schematic of the approach used to derive the BE value associated with the Health Canada tolerable concentration for toluene. The exposure guidance value is based on human data regarding neurological responses in occupationally exposed populations, so no interspecies extrapolations are required. In addition, the monitored biomarker, toluene in blood, was judged to be directly relevant to the likely critical dose metric, toluene concentration in brain. Figure from Aylward *et al*. (2008).

internal dose basis in this case. This results in a BE of 40 μg l^{-1} blood toluene corresponding to the Health Canada TC.

2,4-D

The USEPA Office of Pesticide Programs conducted a recent review of the herbicide 2,4-D and adopted both a chronic oral RfD as well as acute RfDs (applicable to single-day exposures) for 2,4-D (USEPA, 2004). The chronic RfD was derived based on a no-effect level in a chronic rat bioassay with uncertainty factors accounting for interspecies and intraspecies extrapolation (10 each) as well as a database uncertainty factor of 10 to account for certain gaps in the toxicity data.

The pharmacokinetics of 2,4-D have been studied in two sets of human volunteers (Kohli *et al*., 1974; Sauerhoff *et al*., 1977). Both found that 2,4-D is eliminated in urine either as the unchanged parent compound (80–95%) or as a conjugate with urinary half-lives on the order of 1 day. There was no evidence of oxidative metabolism, consistent with data from other mammalian species (Timchalk *et al*. 2004). Under conditions consistent with the definition of an RfD, continuing exposure for more than 1 week of exposure would result in a steady-state in which the amount excreted daily in urine would be approximately equivalent to the amount absorbed each day.

Several mechanisms of action of toxicity for 2,4-D are known, but these are most clearly observed after high exposure acute toxicity events. These include (but are not limited to) dose-dependent cell membrane damage, uncoupling of oxidative phosphorylation, and disruption of acetylcoenzyme A metabolism (Bradberry *et al*., 2000). The specific mechanisms of action underlying the neurotoxicity and other effects observed at lower exposures are not fully understood, and a full discussion of the mechanism of action of 2,4-D is outside the scope of this review. Since 2,4-D does not undergo oxidative metabolism in mammals (reviewed in Timchalk 2004), it is likely that the toxic moiety for most endpoints is the parent compound, 2,4-D.

Because 2,4-D is excreted as the parent compound in urine, most biomonitoring evaluations of exposure to 2,4-D have relied on measurements (quantifying both parent and conjugated compound) in urine samples (Knopp and Glass, 1991; Knopp, 1994; Centers for Disease Control and Prevention, 2005), although a few kinetic studies have examined plasma concentrations of 2,4-D in humans and animals as well (Kohli *et al*. 1974; Saghir *et al*., 2006; van Ravenzwaay *et al*., 2003; Sauerhoff *et al*., 1977). The relative ease of collection of urine samples compared with blood samples contributes to this choice. From a toxicologic point of view, plasma concentrations of 2,4-D are probably more informative for predicting target tissue concentrations and responses (for example, neurotoxic responses). This would be particularly true under conditions of episodic, higher-level exposures. However, under conditions of chronic, low-level exposures, urinary excretion rates should be specific and quantitatively relevant in a framework of a mass-balance assessment. That is, under exposure conditions that approximate steady-state, daily urinary excretion should equal daily intake.

The straightforward elimination kinetics of 2,4-D (as parent compound or conjugate in urine with essentially no oxidative metabolism) and the lack of direct relationship between urinary concentration and critical internal dose metrics suggests a simple mass-balance approach for derivation of BE values for urinary 2,4-D concentration, illustrated in Fig. 4, to estimate the urinary concentration of 2,4-D consistent with the RfD. The process of deriving the BE_{POD} and BE for 2,4-D is summarized below:

- The point of departure (POD) for the USEPA chronic RfD is a NOAEL of 5 mg kg^{-1} day^{-1} in rats fed chronically 2,4-D in the diet. Applying the UF of 10 for interspecies variation, the human equivalent POD is 0.5 mg kg^{-1} day^{-1}. Calculating the concentration of 2,4-D in urine in humans associated with this chronic daily dose yields the BE_{POD}. The daily mass intake at the human equivalent POD was estimated for a variety of child and adult

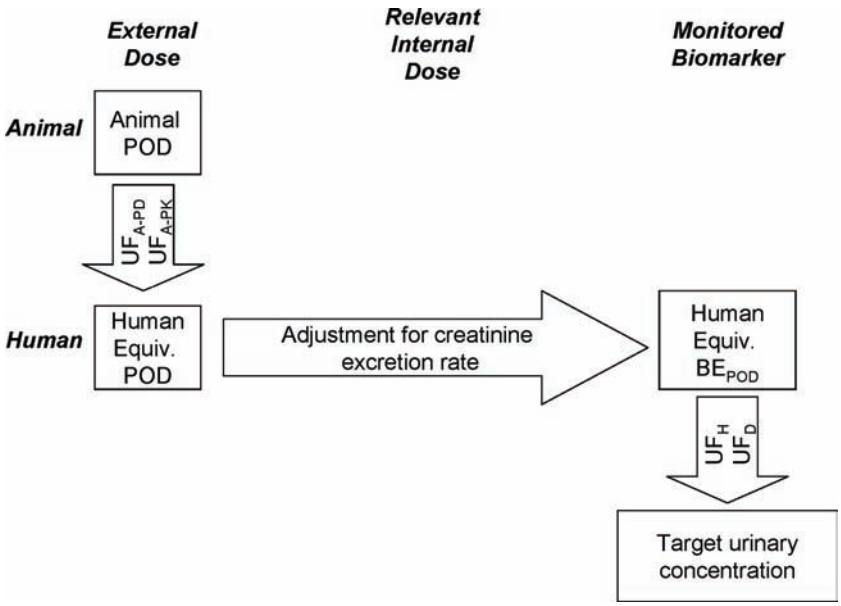

Figure 4. Schematic illustrating the process used to derive the urinary BE value for the USEPA RfD for 2,4-D. The dose level from the animal POD was extrapolated to a corresponding human equivalent POD using conventional UFs as used in the RfD derivation. A urinary mass balance approach was used to derive the corresponding estimated steady-state urinary excretion rate, which was then extrapolated to the final BE value. Figure adapted from Aylward and Hays (2008).

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body weights. Estimated distributions of daily creatinine excretion as a function of sex, age and body size were used in a Monte Carlo analysis to estimate a distribution of creatinine-adjusted urinary 2,4-D concentrations for various age and sex categories (methods are described in detail in Aylward and Hays, 2008). The average of median estimated creatinine-adjusted 2,4-D concentration consistent with chronic exposure at the human equivalent POD for 2,4-D for adults (males and females) is approximately 30 000 $\mu g\ g^{-1}$ creatinine, this value is also consistent with the range of median values identified for children of various ages.

- The BE associated with the chronic RfD is derived by dividing the BE_{POD} by the UF of 10 for intraspecies variation and the UF of 10 for database deficiencies applied by USEPA. The average of the median estimated creatinine-adjusted 2,4-D concentration consistent with chronic exposure at the RfD for 2,4-D for adults (males and females) is approximately 300 $\mu g\ g^{-1}$ creatinine.

Cadmium

Several health-based exposure guidance values and toxicity values are available for cadmium. All non-cancer exposure guidance values have been established to protect against cadmium's effects on the kidney, which is generally identified as the most sensitive endpoint in humans. Cancer risk estimates have also been derived for cadmium by the USEPA and Health Canada to protect against respiratory system tumors resulting from inhalation exposures based on data from occupationally exposed workers. Since it is currently believed that these tumors only occur following inhalation of cadmium dusts, derivation of a corresponding BE is not appropriate because biomonitoring data cannot distinguish the route of exposure (Hays *et al*., 2008b).

Absorption of cadmium from the gastrointestinal tract is estimated to be low, ranging from 0.5 to 12% (average 2%). Cadmium enters the liver, binds to metallothionein and is redistributed to the bloodstream. Because of its small size, cadmium-metallothionein is efficiently transported to the kidney tubules via glomerular filtration (Nordberg *et al*., 2007). Following chronic exposures, kidney has the highest concentrations of cadmium. The biological half-life of cadmium in humans is estimated to range from 6 to 38 years in the kidney and from 4 to 19 years in the liver. Several models of cadmium kinetics in humans have been developed, with varying degrees of complexity and sophistication (International Programme on Chemical Safety, 1992; Kjellström and Nordberg, 1978; Choudhury *et al*., 2001). The value of these models lies in the possibility of using them to calculate relationships between intake and cadmium concentrations in several compartments, including blood and urine, after both short- and long-term exposure (Beckett *et al*., 2007; Nordberg *et al*., 2007).

The advantages of using biomonitoring to assess cumulative exposures to cadmium have been known for decades (Lauwerys and Hoet, 2001). While blood, urine, feces and hair have all been suggested, blood and urine have generally been the biomarkers of choice for assessing cadmium exposures. Generally, urinary cadmium concentrations are believed to be an indicator of chronic exposures, with urinary concentrations reflecting renal cortex cadmium concentrations. In contrast, blood cadmium concentrations are believed to reflect both recent exposure and cadmium in the body accumulated over time (Lauwerys and Hoet, 2001; Alessio *et al*., 1993; Nordberg *et al*., 2007).

The concentration of cadmium in the renal cortex is believed to be the critical dose metric associated with cadmium-induced proteinuria, and urinary cadmium levels have been demonstrated to be highly correlated with renal cortex cadmium concentrations (Orlowski *et al*., 1998; Satarug *et al*., 2002; Nordberg *et al*., 2007). Therefore, urinary cadmium concentration is likely to be a close surrogate for the critical dose metric and thus cadmium-induced proteinuria (Joint WHO/FAO Expert Committee on Food Additives, 2001; American Conference of Governmental Industrial Hygienists, 2001). Given that cadmium concentrations in blood are likely to be more transient in nature than renal cortex levels, blood cadmium concentrations, while still useful, may not be as directly correlated with the critical dose metric associated with the critical toxic response.

All available non-cancer exposure guidance values are based on protecting against kidney toxicity as evidenced by proteinuria (of some form) in humans (reviewed in Hays *et al*., 2008b). These exposure guidance values have identified target renal cortex concentrations with accompanying estimates of corresponding chronic daily intake rates associated with an increased risk of proteinuria in human populations as the POD. Thus, derivation of BE values associated with these exposure guidance values begins with consideration of these POD values. Figure 5 presents a schematic of the approach used to derive the BE values. Briefly, the approach as implemented for the USEPA RfD is as follows:

- Identify the critical dose metric (renal cortex cadmium concentration) associated with the POD. In the derivation of the USEPA RfD, the critica renal cortex cadmium concentration associated with increased risk of urinary proteinuria was identified as 200 $\mu g\ g^{-1}$ in humans.
- Estimate the urinary cadmium concentration (creatinine-adjusted) associated with the critical dose metric (BE_{POD}) using data from studies correlating urinary concentrations with renal cortex concentrations.
- Divide the BE_{POD} by appropriate intraspecies uncertainty factors used in the derivation of the USEPA RfD to derive the BE values.

While detailed pharmacokinetic models exist for cadmium in humans, the primary intended purpose of these models has been to relate external dose to internal concentration of cadmium in tissues (including blood) or in urine. Since the extrapolation needed to calculate the BE for cadmium requires relating renal cortex cadmium concentration with urinary cadmium concentration, more direct empirical relationships (data used in the development of various pharmacokinetic models for cadmium) exist specific to this estimation. Several studies have measured cadmium concentrations in both renal cortex and urine in both deceased and living patients (Orlowski *et al*., 1998; Satarug *et al*., 2002), thus provide robust and direct data for estimating the urinary concentration associated with the target renal concentration. The BE and BE_{POD} associated with USEPA's RfD are 2 and 6.3 $\mu g\ Cd\ g^{-1}$ creatinine, respectively.

Acrylamide

The USEPA established a RfD for acrylamide in 1991 (a revised USEPA risk assessment was released in draft form in 2008, but the BE value described here is based on the older RfD value) based on the most sensitive endpoint of nerve damage in rats exposed subchronically to acrylamide in drinking water (USEPA,

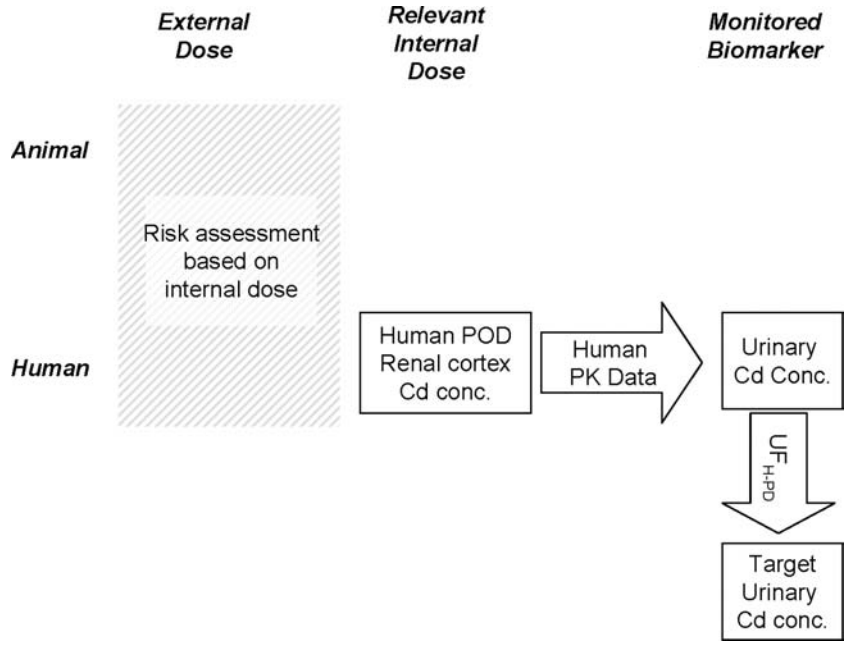

Figure 5. Schematic illustrating the derivation of the cadmium urinary BE value corresponding to the USEPA RfD. The critical renal cortex concentration identified by USEPA as the POD for the derivation was extrapolated to a corresponding urinary concentration using available autopsy data. Appropriate intraspecies UFs were then applied to derive the BE value. Figure adapted from Hays *et al.* (2008c).

2007a). Additional exposure guidance values, including cancer slope factors are reviewed in the acrylamide BE Dossier (Hays and Aylward, 2008). Acrylamide (AA) is readily and rapidly absorbed following oral ingestion. Once absorbed, AA either reacts with glutathione to form *N*-acetyl-*S*-(2-carbamoylethyl)cysteine (AAMA: considered a deactivation pathway) or is metabolized via CYP2E1 to form glycidamide (GA, considered an activation pathway). The extent of glutathione conjugation and conversion to glycidamide varies among species (Shipp *et al.*, 2006), making some species more susceptible to AA toxicity. GA also reacts with glutathione to form mercapturic acids *N*-acetyl-*S*-2-(2-hydroxy-2-carbamoylethyl)cysteine (GAMA) and *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)cysteine. AA, GA and the mercapturic acids are predominantly excreted in the urine, and several of these moieties have been measured as biomarkers for exposure to AA. Both AA and GA form adducts with sulfhydryl groups on hemoglobin, proteins and with DNA (Shipp *et al.*, 2006). The hemoglobin adducts of AA and GA are also commonly used as biomarkers for exposure to AA.

While the tumor response has been linked with the GA metabolite (Ghanayem *et al.*, 2005) and is likely associated with the area under the GA serum curve (Shipp *et al.*, 2006), the relevant internal dose metric for neurotoxicity is less well defined in terms of the mechanism of action, but is probably associated with AUC or peak serum AA, GA or both (Shipp *et al.*, 2006). The concentration of AA and GA in serum are the most relevant internal dose metrics for purposes of deriving a BE, however, both AA and GA have short half-lives in serum (on the order of several hours). Hemoglobin adducts of AA and GA, in contrast, provide an integrated index of exposures to AA since the rate of removal of hemoglobin adducts appears to be limited by the rate of red blood cell (RBC) turnover, about once every 120 days.

The derivation of BE values for hemoglobin adducts of AA and GA corresponding to the USEPA oral RfD relies upon extrapolation from animals to humans on the basis of serum concentrations of AA and GA (Hays and Aylward, 2008). The steps are illustrated in Fig. 6 and described briefly below.

- *Estimate relevant internal dose metrics (AA and GA serum concentrations) in rats*. Several groups have measured the serum concentrations of AA and GA in F344 rats following a single 30 min 0.1 mg kg^{-1} dietary dose of AA (Doerge *et al.*, 2005a) and following chronic (1 mg kg^{-1} d^{-1}) drinking water exposures (Tareke *et al.*, 2006; Doerge *et al.*, 2005b). The daily average serum concentrations of AA and GA in rats following a single dose of AA in diet and following three weeks' exposure to AA in drinking water are remarkably similar when normalized to dose, yielding average serum concentrations of both AA and GA of 0.65 μM in blood per mg kg^{-1} day^{-1} administered dose. These data indicate that the relationship between daily dose and average serum concentrations of AA and GA is linear in the tested dose range (0.1–1.0 mg kg^{-1} day^{-1}) in rats. The NOEL used as the basis of the RfD derivation (0.2 mg kg^{-1} day^{-1}) falls into this range as well. Therefore, the relevant internal dose associated with the POD used in the RfD derivation can be estimated as approximately 0.13 μM AA or GA in serum (steady-state average concentration).
- *Apply relevant duration and interspecies uncertainty factors*. In the derivation of the RfD, the USEPA applied an uncertainty factor of 10 for subchronic to chronic exposures, and this uncertainty factor was maintained in the derivation of the BE values. The human equivalent POD is calculated by dividing by another factor of $10^{0.5}$ to account for species differences in pharmacodynamics. The typical uncertainty factor component of $10^{0.5}$ used to account for species differences in pharmacokinetics is explicitly accounted for by using the relevant internal dose. Therefore, the steady-state concentrations associated with the human equivalent POD are 0.004 μM AA or GA in serum.

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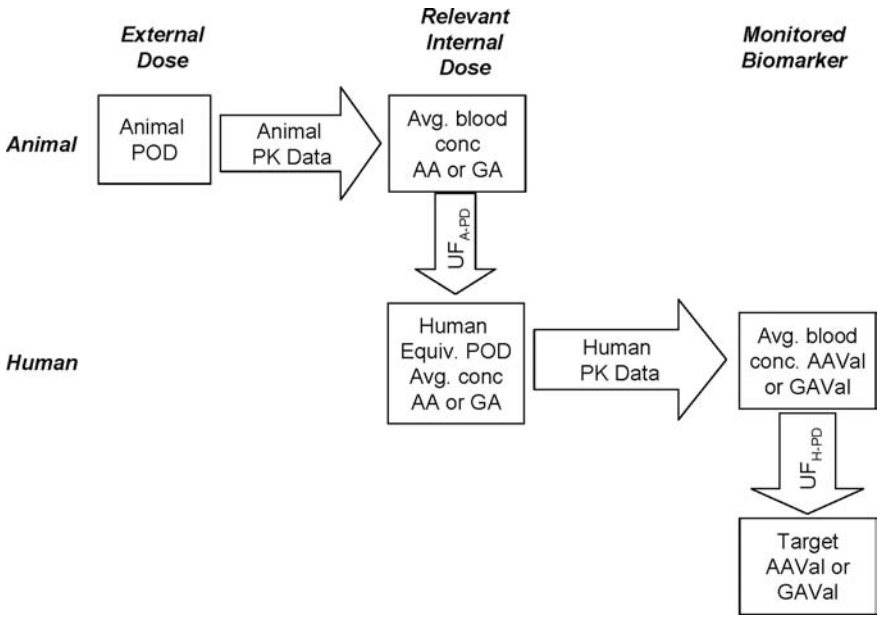

Figure 6. Schematic illustrating the derivation of the acrylamide BE value associated with the USEPA RfD. Key steps in the derivation include the estimation of rodent serum AA and GA concentrations at the POD based on experimental data and estimation of the steady-state hemoglobin adduct levels of AAVal and GAVal based on *in vitro* reaction rate data (see text). Figure from Hays and Aylward (2008).

- *Estimate steady-state AAVal and GAVal levels associated with target serum AA and GA AUC concentrations in humans*. The next step in developing BEs for acrylamide involves relating the steady-state serum AA and GA daily AUC to the expected chronic steady-state concentrations of AAVal and GAVal in humans. The most direct method for this extrapolation involves knowing the rates of AAVal and GAVal formation (as a function of serum AA and GA AUCs) and the rates of AAVal and GAVal removal (as a function of RBC turnover) in humans. These data have been developed in humans (Fennell *et al*., 2005; Bergmark *et al*., 1993). Using the rates of AAVal and GAVal adduct formation reported by Fennell *et al*. (2005) and an RBC lifespan of 120 days (Osterman-Golkar *et al*., 1976), the resulting BE_{POD} values are 25.3 fmol mg^{-1} globin and 39.8 fmol mg^{-1} globin for AAVal and GAVal, respectively.
- *Apply appropriate intraspecies uncertainty factor components*. The BEs are calculated by dividing the BE_{POD} by an additional factor of $10^{0.5}$ to account for human variability in pharmacodynamics. The additional component of $10^{0.5}$ typically used to account for human variability in pharmacokinetics are not applied since the biomarkers are directly related to the relevant internal dose metric.

The resulting BE values associated with USEPA's RfD are 8 and 13 fmol mg^{-1} globin for AAVal and GAVal, respectively (Hays and Aylward, 2008).

Interpretation of Biomonitoring Data using BE Values

Biomonitoring data from the general population can be compared with the BE values derived for each of the case study compounds listed to illustrate the use of BEs in interpretation of such data. Figure 7 illustrates the BE and BE_{POD} values associated with the Health Canada inhalation tolerable concentration for toluene as well as the 95th percentile from a study of blood toluene levels in school-aged children in Minneapolis following repeated measures over the course of two years (Sexton *et al*., 2005). The measured values are more than 100-fold lower than the BE value, placing these results in the category of low-priority for risk assessment follow-up. A number of caveats and limitations must be conveyed when making such comparisons, however. The BE values, like the underlying exposure guidance values, are not bright lines between safe and unsafe levels and do not represent diagnostic criteria indicating the presence or absence of an adverse effect. The biomonitoring data collected in a given study, particularly for relatively short-lived compounds such as toluene, do not necessarily represent long-term exposure levels.

The BE values have their greatest utility as a tool for prioritization of risk assessment and management efforts. Biomonitoring data showing the detection of chemical compounds in human blood or urine provoke powerful reactions in the public, particularly when there is no framework for interpretation of the measured values in a health risk context. Risk management efforts may be focused on a chemical simply because it is detected, or because the chemical is considered to be 'toxic'. However, potential health risks are a function of both hazard and exposure. Human biomonitoring data, which provide a snapshot of exposure integrated from all sources, coupled with interpretation using BE values, provides an opportunity to include a health risk perspective, based on current risk assessments for the chemicals, in decisions regarding chemical risk management efforts.

The ratio between the BE value and the measured values in a given biomonitoring study can be regarded as a 'margin of safety' (MOS). When levels of multiple chemicals are measured in a population, estimation of the chemical specific MOS values can serve as a prioritization tool for risk managers, providing one basis for focusing efforts and resources on chemicals with the lowest MOS. Table 1 illustrates this effort in practice using the BE values for the case study compounds described here and recent data from general population biomonitoring studies. The

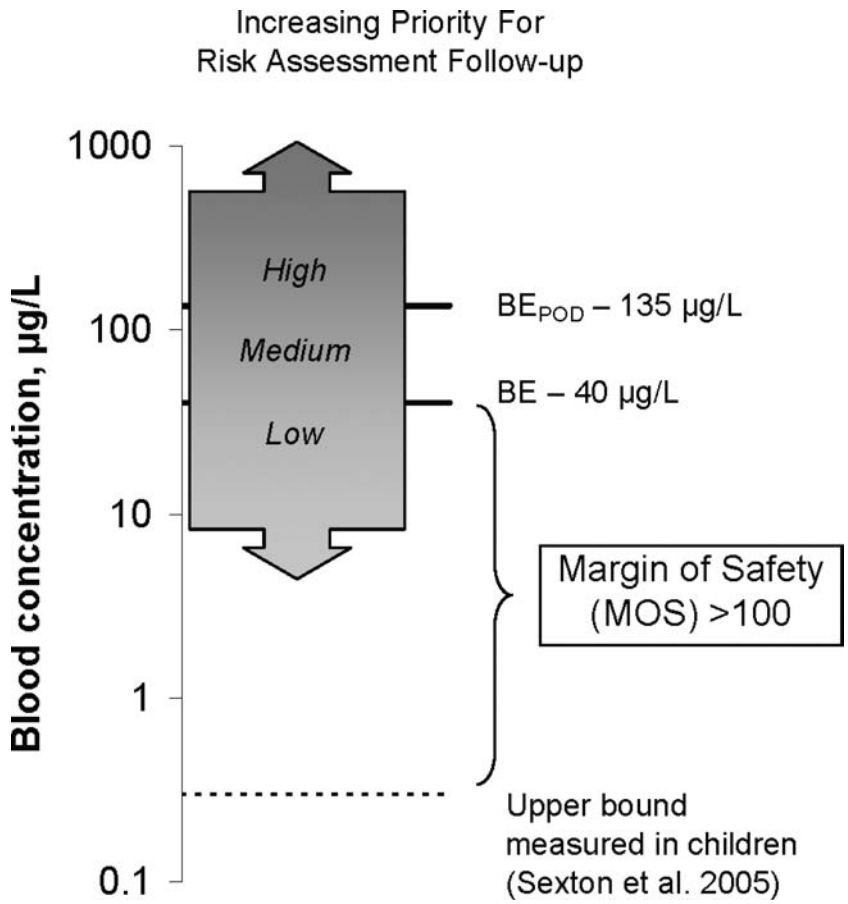

Figure 7. Interpretation of toluene biomonitoring data in the context of the toluene BE. The presentation of biomonitoring data and results in the context of the BE values should include key communication messages: (a) BE values are screening values. They can be used to provide a screening level assessment of measured concentrations of toluene in blood in population- or cohort-based studies to assess whether the measured values in a given study are of low, medium or high priority for risk assessment follow-up. (b) BE values are not diagnostic criteria or bright lines between 'safe' and 'unsafe' levels. They cannot be used to evaluate the likelihood of an adverse health effect in an individual or even among a population. Measured concentrations of toluene in blood may not be representative of long-term exposure levels.

Table 1. Comparison of selected BE values for the case study compounds with current biomonitoring data for four compounds with resulting margins of safety (MOS) and relative priority for risk assessment follow-up, as described in the guidelines for BE communication (LaKind *et al*., 2008)

Chemical	Exposure guidance value	BE	Measured biomarker concentrations	MOS	Priority for risk assessment follow-up
Toluene	Health Canada Inhalation Tolerable Concentration	40 µg l^{-1} toluene in blood	Median: 0.1 µg l^{-1} in blood 95th percentile < 0.4 µg l^{-1} in blood[a]	>100	Low
2,4-D	USEPA RfD	300 µg 2,4-D g^{-1} creatinine in urine	95th percentile ~1 µg g^{-1} creatinine in urine[b]	~300	Low
Cadmium	USEPA RfD	2 µg Cd g^{-1} creatinine in urine	95th percentile ~1 µg g^{-1} creatinine in urine[b]	~2	Low to medium
Acrylamide	USEPA RfD	8 fmol mg^{-1} globin AAVal	20–70 fmol mg^{-1} globin[c]	<1	Medium to high

[a]Sexton *et al*. (2005) — repeated measurements of blood concentrations in school aged children in Minneapolis.
[b]Centers for Disease Control and Prevention (2005) — data from the NHANES 2001–2002 study in the US general population.
[c]Hagmar *et al*. (2001) — data from a small sample from the German population.

measured levels are designated as indicating relative priority for risk assessment follow-up, in accordance with the communication guidelines developed in the BE expert workshop (LaKind *et al*., 2008).

Note that the evaluation presented in Table 1 relies upon BE values derived for a single exposure guidance value for each of these four compounds. Additional exposure guidance values exist for each compound and will provide different estimates

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of the MOS. In addition, not all of the biomonitoring data sets listed in the table are based on population-representative data. Finally, for many compounds, exposures may be transient or change over time, so the conclusions regarding the measured biomarker concentrations must be evaluated in terms of potential temporal variations. However, even with these limitations, the biomonitoring data in the context of the BE values provide a relatively clear picture, with two compounds, cadmium and acrylamide, demonstrating a far narrower margin of safety in the context of the existing risk assessments for these compounds, and therefore being designated as having higher priority for risk assessment follow-up.

An alternative approach to use of the BE values evaluates the ratio between the BE_{POD} (biomarker concentration associated with the human equivalent point of departure) and measured biomarker levels to estimate a 'margin of exposure' (MOE). This approach is analogous to approaches often used to evaluate chemical exposure levels in the European Union (Barlow *et al*., 2006). However, unlike typical margin-of-exposure evaluations, which compare external exposures to a point of departure (such as a NOAEL) from animal studies, the BE_{POD} is already set at a level that includes interspecies extrapolation uncertainty factors. In addition, intraspecies variability in pharmacokinetics will be reflected in the measured biomarker concentrations. These differences between a conventional, external exposure-based assessment of MOE and the proposed approach using the BE_{POD} needs to be clearly recognized when conducting such MOE comparisons, and the typical targets for margin of exposure will probably need to be reconsidered to take these factors into account.

Screening large sets of biomonitoring data with measurements of multiple analytes will require derivation of additional chemical-specific BE values. As BEs are derived for increasing numbers of compounds, the value of such human biomonitoring data for population risk evaluation, prioritization and management will increase.

Future Challenges

The use of biomonitoring to measure trace human exposures to chemicals in the environment and from consumer products has gained increased attention and utility, despite the lack of tools to interpret the data in a health risk context. As BEs are increasingly developed and used to interpret biomonitoring data, this will help to focus attention on the chemicals that warrant continued and more focused monitoring and also will provide a tool for prioritization of risk management efforts.

The guidelines for the derivation of BEs are very similar to the current practice being employed to derive exposure guidance values that are based on mode of action and internal dose metrics. Increasingly, as the risk assessment process has evolved, exposure guidance values are starting to be developed more with just such a focus — on mode of action and internal dose metrics. Therefore, as part of this evolution, there is a natural fit for the BEs to be developed along with such exposure guidance values. For example, the recent risk assessment from the USEPA for 1,1,1-trichloroethane (USEPA, 2007b) focused on data regarding neurological effects from studies of human volunteers exposed to known air concentrations. These studies also included values of blood concentrations at the point of departure used in the risk assessment. These data form a natural basis for the development of a BE value corresponding to the RfC.

For some compounds being biomonitored, there are no existing exposure guidance values, even though there may be toxicity and pharmacokinetic data available. There are several possible approaches for such situations. One approach might involve selecting a POD from available toxicology studies (even if the studies have some limitations from a toxicological standpoint) and a standard set of conservative uncertainty factors to derive a provisional BE_{POD} and BE. Another approach could leverage the Threshold of Toxicological Concern (TTC; Kroes *et al*., 2000) or other similar approaches to derive BEs for a class of compounds. In some cases there may be an exposure guidance value, but pharmoacokinetic data could be lacking. In such instances application of a generic PBPK model (Liao *et al*., 2007) may provide the means to develop a provisional BE. In such instances, these types of provisional BEs could be used as place holders until a thorough chemical risk assessment is conducted and exposure guidance value is derived. The provisional BE approach could also help to inform decisions regarding which chemicals might warrant priority attention for such risk assessment efforts.

Derivation of a BE involves a careful evaluation of the mode of action and the most relevant internal dose metric and the biomarkers that are most closely related to the relevant internal dose metric. This effort helps to identify which biomarkers are the most easily interpretable from a health risk context for the individual compound. As such, sometimes the most easily interpretable biomarker (from a toxicological point of view) does not coincide with the most convenient medium to collect (blood vs urine) or the analyte most easily quantified. When there is this discrepancy, it may help focus researchers as to which biomarker to sample. Conversely, such a discrepancy may help drive research to help reduce uncertainties associated with interpreting certain biomarkers from a health risk standpoint. There are other situations that will arise in which the biomarker commonly sampled is not specific to the compound of interest. While this approach has value in the workplace (because exposures in the workplace are higher and thus background exposures to other compounds not of interest do not interfere or contribute as greatly), this becomes a problem for interpreting biomonitoring data from general environmental exposures. The BE approach helps identify such situations and focus research efforts on issues that will enhance the value of collected biomonitoring data.

Another advantage of the BE is that it provides a range of chemical-specific biomonitoring levels of interest. This may be helpful in the design of biomonitoring-based studies, which must achieve a balance between detection limit desired, volume of biological sample needed and analytical costs associated with lower limits of detection. The BE provides a benchmark to identify the range of biomarker concentrations that may be of interest and therefore a means to target a limit of detection. If the current limits of detection are far below the BE, resources may be saved by raising the limit of detection and still providing sufficient context for interpretation. Alternatively, if the achievable limits of detection are far above the BE and/or BE_{POD}, then the analytical data may not be useful in the context of examination of lower-level exposures that are relevant in the risk assessment context.

Conclusions

Biomonitoring Equivalents provide a practical and flexible approach to interpreting human biomonitoring data in a public health risk context by leveraging existing chemical risk assessments and

existing pharmacokinetic data in animals and/or humans. By the nature of both the underlying risk assessments and the likely limitations in available data, BEs must be regarded as screening tools with an inherent level of uncertainty associated with them. However, the BE approach serves as a rational, science-based first step that has many practical advantages. The BE process does not seek to replace the existing risk assessments for chemicals, but rather seeks to translate those risk assessments so that biomonitoring data can be interpreted in the context of those existing risk assessments. The BE approach provides a framework for potentially improving risk assessments by focusing on mode of action and relevant internal dose metrics and provides a useful framework for identifying the most relevant chemical-specific biomarker(s). As more BEs become available, a major utility of the BEs will be for risk prioritization and identifying which chemicals warrant more attention compared with those chemicals which have sufficient margins of safety.

Other approaches to interpretation of biomonitoring data in the context of current risk assessments include the application of 'reverse dosimetry' modeling in order to identify a possible distribution of external exposure levels that led to an observed distribution of measured biomarker concentrations in a population (Clewell *et al*., 2008; Georgopolous *et al*., 2009; Liao *et al*., 2007; Tan *et al*., 2007). As noted by numerous authors, the reverse dosimetry approach is computationally intensive, is data-set specific (a new reverse dosimetry modeling exercise is required for every set of biomonioring data) and is an attempt to address probabilistically what is an infinite-solution problem (Rigas *et al*., 2001; Georgopolous *et al*., 2009). That is, an infinite number of exposure scenarios and time courses can lead to a measured biomarker concentration in an individual at a specific point in time. The BE approach provides a screening-level alternative to these complex, computationally intensive efforts by leveraging the inherent value of biomonitoring data: measured biomarker concentrations provide a snapshot of exposure that is more directly relevant to potential toxicological response than measures of external exposure. Rather than 'back-calculate' possible *external* exposure levels that led to each measured biomarker concentration (and thus introducing uncertainty through the multiple assumptions that are included in such a process), the BE approach instead compares those measured levels to biomarker concentrations that are consistent with existing risk assessments. If those comparisons show a low margin of safety for a given chemical, detailed exposure pathway and time course investigations (such as those required to conduct reverse dosimetry exercises) can be pursued. However, if the biomonitoring data for a chemical show a wide margin of safety, such resource-intensive efforts can be directed to other chemicals with lower margins of safety.

Some important lessons have been learned during the process of the BE values for the case study chemicals. Because BE values and the measured biomarker levels are both based on internal dose measures, fully developed PBPK models, which ultimately focus on relating internal dose metrics to *external* doses, are often not necessary. Of the case study chemicals, a fully developed PBPK model was used only for toluene. For the other chemicals, simpler approaches were available based on available data sets in the literature that directly relate internal dose measures to the toxicological effects of interest. In addition, because the BE approach focuses on estimating steady-state biomarker levels consistent with exposure guidance values, transient target tissue concentrations associated with complex dosing regimens do not need to be replicated. Simple pharmacokinetic relationships available from the literature, assumption of urinary excretion mass balance, and data sets correlating measured blood concentrations in animals or humans with relevant administered doses can be used to estimate biomarker concentrations at the point of departure. Lastly, in situations where no available pharmacokinetic data exist for calculating a BE, a relatively simple pharmacokinetic study can be designed and executed in the species of interest to measure blood concentrations associated with the dosing regimen at the POD (see, for example, Saghir *et al*., 2006). Such data have been recommended for routine inclusion in toxicity testing protocols (Barton *et al*., 2006) and can be leveraged to estimate BE values.

As more BE values are developed, more lessons will undoubtedly be learned. Likewise, as BE values and biomonitoring data become more widely available, more applications in the public health and regulatory arenas will be identified. Having a metric to interpret human biomonitoring data will also help to expand the arenas where biomonitoring data itself will be useful. It is likely that having a metric like BEs will spur biomonitoring studies of target populations with special exposures and populations that may be sensitive because of differences in pharmacokinetics. The future will obviously identify new opportunities and challenges for the application of BEs and will undoubtedly identify modifications to the BE guidelines for derivation and communication.

Acknowledgements

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