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Guidelines for the derivation of Biomonitoring Equivalents: Report from the Biomonitoring Equivalents Expert Workshop

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ABSTRACT

Biomonitoring Equivalents (BEs) are defined as the concentration of a chemical (or metabolite) in a biological medium (blood, urine, human milk, etc.) consistent with defined exposure guidance values or toxicity criteria including reference doses and reference concentrations (RfD and RfCs), minimal risk levels (MRLs), or tolerable daily intakes (TDIs) [Hays, S.M., Becker, R.A., Leung, H.W., Aylward, L.L., Pyatt, D.W., 2007. Biomonitoring equivalents: a screening approach for interpreting biomonitoring results from a public health risk perspective. Regul. Toxicol. Pharmacol. 47(1), 96–109]. The utility of the BE is to provide a screening tool for placing biomonitoring data into a health risk context. A Panel of experts took part in the Biomonitoring Equivalents Expert Workshop to discuss the various technical issues associated with calculating BEs and developed a set of guidelines for use in the derivation of BEs. Issues addressed included the role of the point of departure (POD) in BE derivation, the appropriate application of human and animal kinetic data and models, consideration of default uncertainty factor components in the context of internal dose-based extrapolations, and relevance of mode of action to technical choices in kinetic modeling and identification of screening values. The findings from this Expert Panel Workshop on BE derivation are presented and provide a set of guidelines and considerations for use in BE derivation.

Abbreviations: AAMA, *N*-acetyl-*S*-(2-carbamoylethyl)cysteine (a glutathione conjugate metabolite of acrylamide); ACGIH, American Conference of Governmental Industrial Hygienists; ATSDR, Agency for Toxic Substances and Disease Registry; AUC, area under the curve; BE, Biomonitoring Equivalent; BEI, biological exposure index; BE_{POD}, Biomonitoring Equivalent associated with the point of departure; BE_{POD_Animal}, Biomonitoring Equivalent associated with the point of departure in the animal study underlying the exposure guidance value; FMV, first morning void; LED_{10}, lowest effect dose corresponding to a 10% increase in response rate; LOAEL, lowest observed adverse effect level; MOE, margin of exposure; MRL, minimal risk level; NOAEL, no observed adverse effect level; PBPK, physiologically based pharmacokinetic; POD, point of departure; RfC, reference concentration; RfD, reference dose; RSD, risk-specific dose; TDI, tolerable daily intake; TTC, thresholds of toxicological concern; 2,4-D, 2,4-dichlorophenoxyacetic acid; WHO, World Health Organization; UF, uncertainty factor; $UF_{A\text{-}PD}$, uncertainty factor accounting for inter-species differences in pharmacodynamic responses; $UF_{A\text{-}PK}$, uncertainty factor accounting for inter-species differences in pharmacokinetics; $UF_{H\text{-}PD}$, uncertainty factor accounting for within human variability in pharmacodynamic responses; $UF_{H\text{-}PK}$, uncertainty factor accounting for within human variability in pharmacokinetics; USEPA, United States Environmental Protection Agency.

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1. Introduction

Interpretation of occupational biomonitoring data in a health risk context has a substantial history (Yager, 1990; Rappaport et al., 1995; Morgan, 1997; Fiserova-Bergerova, 1987, 1990). A framework has been lacking, however, for the interpretation of biomonitoring data from the general public's exposure to environmental chemicals. The concept of the Biomonitoring Equivalent (BE) presented in Hays et al. (2007) is an approach for using available pharmacokinetic data and forward dosimetry to calculate levels of biomarkers anticipated to be associated with exposures consistent with general population exposure guidance values such as reference doses (RfDs), minimal risk levels (MRLs), and tolerable daily intakes (TDIs) and their underlying toxicological points of departure (PODs)[1] as a basis for putting biomonitoring data into a public health risk context (Fig. 1). Hays et al. (2007) recognized that BEs in their simplest definition are a basic, screening level approach for putting biomonitoring data into a health risk context (Fig. 2). Screening can be defined as the application of simple tools or procedures that can be applied rapidly to delineate populations that may be at some degree of increased health risk from those that may not. Depending on outcome, screening procedures require detailed confirmatory follow-up before definitive conclusions can be reached. Along a continuum of increasing sophistication (and data requirements), BEs are more sophisticated than generic screening criteria analogous to thresholds for toxicological concern (TTC) (Kroes et al., 2004). However, the BE approach (in its simplest form) is less sophisticated than a comprehensive internal dose-based risk assessment, which in turn may have greater uncertainties than biomonitoring interpretation tools that are based on human biomonitoring-based epidemiology (for example, blood lead screening). The BE leverages existing exposure guidance values and existing information on pharmacokinetics in animals or humans to convert an existing exposure guidance value and POD into a biomarker level. The internal dose-based risk assessment approach relies on quantitatively relating the POD in animals (or humans) to a critical dose metric (the tissue concentration of the active chemical form causing the toxicity) that then is used as the metric to scale to a tolerable exposure level in humans. The human epidemiology-biomonitoring derived standards rely on developing a quantitative understanding of the relationship between biomonitoring levels in humans and an observed biological/toxicological response (an example is the US Centers for Disease Control level of concern established for lead of 10 μg/dL [or 100 μg/L] blood). The approaches have commonality with the methods identified in the National Research Council report on the interpretation of biomonitoring (NRC, 2006). The closer or more relevant the biomarker is to the critical dose metric, the more closely aligned with an internal dose risk assessment the BE may become, and the less uncertainty may be associated with use of the BE value for interpreting human biomonitoring data (Hays et al., 2007). In such cases, consideration may be given to replacing default UF components for pharmacokinetic variability with modeling approaches, similar to approaches previously used in risk assessments for compounds with well-developed physiologically based pharmacokinetic (PBPK) models (see, for example, USEPA, 1999).

There are several approaches available for developing BEs which vary in sophistication and robustness (Hays et al., 2007). Numerous specific issues and options likely to be encountered in the derivation of BEs were identified, including:

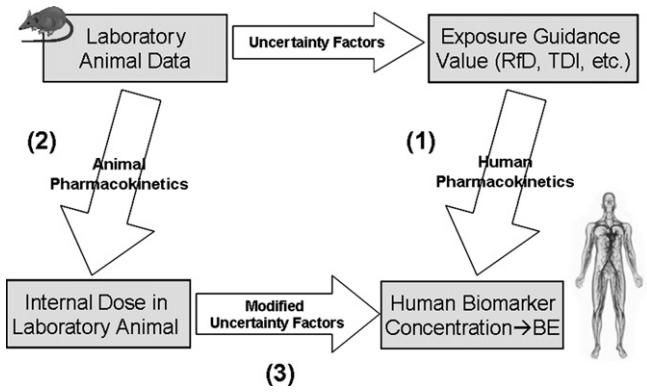

Fig. 1. Schematic diagram showing parallelogram concept for calculating BEs and possible routes for deriving a BE.

- What types of exposure guidance values should be used as the basis for derivation of BE values?
- Is it better to start with animal pharmacokinetic information and calculate biomarker concentrations associated with the POD and then transform to a BE by applying appropriate uncertainty factors (UFs), or is it more scientifically defensible to simply calculate the BE in humans as a direct translation of exposures at the exposure guidance value (see Fig. 1)?
- Does use of internal dose metrics suggest or require the replacement of default UFs associated with inter- or intra-species pharmacokinetic variability with modeling approaches?
- Do exposure guidance values set for short-lived and/or long-lived biomarkers pose unique challenges that should be addressed through special approaches for calculating BEs?

An Expert Panel was assembled to consider the technical issues inherent in the derivation of BE values. The objective of the Derivation Workshop was to develop guidelines to inform the selection of data and approaches for deriving Biomonitoring Equivalents (BEs). The Expert Panel's evaluations and responses to charge questions served as the basis for this guidelines paper.

Communication of BEs is an important aspect and should help guide the derivation of BEs. As a result, the Expert Panel included members with expertise in risk communication, ethics, and medicine who took part in the deliberations and provided context about how decisions in deriving BEs impact the communication of BEs and their utility for communicating the interpretation of biomonitoring data in a health risk context.[2]

The BE concept, as initially proposed, focused on a relatively simple translation of existing health-based exposure guidance values such as RfDs, reference concentrations (RfCs), MRLs and TDIs into estimated biomarker concentrations (Hays et al., 2007). However, the use of pharmacokinetic data to estimate internal dose metrics associated with external doses requires consideration of the extrapolation process used to derive the exposure guidance values, beginning with the underlying POD. Further, the BE approach provides the opportunity to replace default uncertainty assumptions with chemical-specific information, depending upon the relationship between the biomarker and critical dose metric. As a result, the focus shifted toward a more fundamental, internal dose-based risk assessment approach for the BEs, starting with the POD from the animal toxicology study (or human toxicity data)

[1] The dose–response point that marks the beginning of a low-dose extrapolation. This point is most often the upper bound on an observed incidence or on an estimated incidence from a dose–response model (http://www.epa.gov/iris/gloss8_arch.htm#p).

[2] The communication panel also had charge questions that guided their own deliberations. The results of those deliberations are included in the companion paper (LaKind et al., 2008).

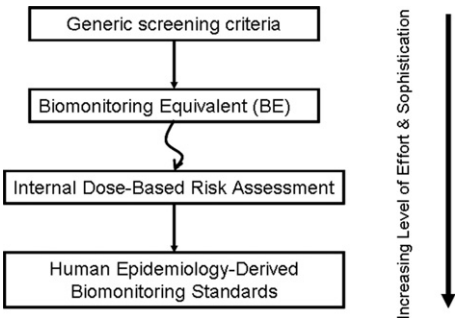

Fig. 2. Sophistication continuum of biomonitoring screening and interpretation tools. Generic screening criteria analogous to thresholds of toxicological concern (TTCs; Kroes et al., 2004) could conceivably be developed without use of chemical-specific information. BEs use available chemical-specific pharmacokinetic information, but limited information on internal dose–response and mechanism of action. A comprehensive internal dose-based risk assessment would rely on more complete understanding of those factors, while standards derived directly from biomonitoring-based epidemiological data (for example, the blood lead standard) represent the most sophisticated biomonitoring interpretation tools. Along with the increasing level of sophistication is a requirement for increasing resources and information for development.

underlying a given exposure guidance value. This shift in focus grew out of several considerations, including the goal of increased transparency in the derivation; the potential for harmonization of BE values derived from exposure guidance values established by different agencies; the potential for harmonizing the approach to derivation of cancer and non-cancer based values; and the ability to incorporate a margin of exposure (MOE) framework in the use of BE values in the interpretation of biomonitoring data. These considerations are discussed in more detail throughout this Workshop report.

2. Starting points for BE derivation

The Panel considered in detail the initial steps involved in deriving BE values. In particular, the following topics were discussed:

- Selection of exposure guidance values;
- Selection of analytes;
- Pharmacokinetic data requirements; and
- Point of departure (POD) underlying the derivation of the exposure guidance value(s) as the major focus of derivation.

The Panel's evaluation of each of these topics is discussed further below.

2.1. Exposure guidance value selection

Exposure guidance values designed for protection of the general population, including sensitive subpopulations under chronic exposure conditions, are appropriate to use as starting points for BE derivation. These include RfDs and RfCs from the US Environmental Protection Agency (USEPA), MRLs from the US Agency for Toxic Substances and Disease Registry (ATSDR), and TDIs from the World Health Organization (WHO) or Health Canada. When using these values, evaluations should focus first on the POD selected as the basis for the derivation of the associated exposure guidance value(s) for reasons discussed further below. Preference would be given to exposure guidance values based on more recent toxicological evaluations, which are more likely to consider all relevant data, but it may also be important to use values applicable to the country, geographic location, or population for which the BE will be used.

Exposure guidance values specific to intermediate or acute duration exposures could be used as the basis for BE derivation, with the provision that such BE values be applied only to biomonitoring data generated under comparable exposure situations. For example, a BE value derived based on an acute duration exposure guidance value would be appropriately applied to evaluate biomonitoring data collected following an acute exposure event.

BEs derived from occupational standards might be useful or appropriate for interpretation of biomonitoring data from occupationally exposed individuals, but they cannot simply be used for interpretation of biomonitoring data from the general population because, workplace exposure standards usually have not been developed with the additional safety factors used to set exposure guidance values to protect the health of the general population, including susceptible populations such as children, pregnant women and the elderly. The American Conference of Governmental Industrial Hygienists (ACGIH) develops values similar to BEs, called biological exposure indices (BEIs) for selected chemicals. The BEIs typically would be applied by occupational health groups for analyzing biomonitoring data from exposed workers.

Exposure guidance values based on technological rather than health considerations (for example, exposure guidance values that are set to accommodate best available control technology, or chemical detection limits, etc.) are not an ideal basis for BE development, because biomonitoring data either below or above BEs for such values cannot be interpreted in a health risk context, but only, potentially, in an exposure context. Exposure guidance values that are established to protect route of entry effects present additional issues in the interpretation of biomonitoring data. For instance, some RfCs may be established to protect from pulmonary effects and some RfDs may be established to protect against gastrointestinal effects due in both cases to local effects at the site of entry into the organism. Since biomonitoring data cannot distinguish among the routes of entry for chemical exposures, BEs derived from exposure guidance values established to protect against route of entry effects could be misleading unless the exposures for a given chemical are known to occur predominantly by the route of entry of concern.

2.2. Target analyte selection

The same considerations that drive the selection of a target analyte for BE derivation are generally also factors in the design of biomonitoring studies and health surveillance programs. For any given chemical there could potentially be multiple BEs derived for different analytes (e.g., parent and metabolites) in different biological matrices (e.g., blood, urine, hair). From a practical point of view, the primary consideration in the selection of the target analyte should be to identify the combination of analyte(s) and biological matrix associated with a specific chemical exposure for which analytical methods already have been developed. BE values based on such analytes would have the most utility in interpretation of existing biomonitoring data. However, additional considerations should help guide the selection of target analytes for development of BEs to best facilitate the interpretation of biomonitoring data in a health risk context, including:

- *Specificity*. Where possible, the analyte should be a specific marker of exposure to the chemical of interest. From the toluene case study (Aylward and Hays, 2008) two urinary markers for toluene used in the occupational setting, ortho-cresol and hippuric acid, are non-specific to toluene and of limited use at environmental exposure levels, while toluene in blood is a specific biomarker.

- *Relevance to toxicity*. Where information is available to guide the decision, analytes should be selected that are most relevant to the toxic endpoint of interest. For example, toluene in blood is directly relevant to nervous system responses, the most sensitive responses observed in humans following inhalation exposures.
- *Relevance to exposure*. In some instances, available analytes most directly relevant to toxicity (such as a minor, but toxic metabolite) may be poorly related to exposure, and may thus have limited applicability in the interpretation of body concentrations relative to specific levels of exposure. In this instance, depending upon the purpose of the BE and the availability of various analytes, one may wish to develop a BE for a biomarker closer to exposure, closer to toxicity, or perhaps both.
- *Stability of analyte*. In some instances, the parent compound or active moiety is short-lived. Where possible and informative, analytes that are more stable should be targeted. For example, some urinary metabolites of short-lived compounds are longer-lived and could provide a time-integrated indication of exposure (but would not provide information on peak concentrations at the critical tissue). In the case of acrylamide (Hays and Aylward, 2008), hemoglobin adducts provide a more persistent marker of acrylamide exposure than parent compound in blood.
- *Acceptability*. Biomarkers in media requiring a less-invasive collection procedure (for example, hair or urine) may be preferred, and any cultural or ethnic considerations may also affect selection of biological media.
- *Ease of interpretation*. The process of developing a BE also would likely require identifying the most relevant and easily interpretable biomarker from a health risk context.

The description of the BE derivation should include recommendations for the optimal biomarkers (analyte and matrix) and also include a discussion of the considerations in the choice of the biomarker in the documentation.

2.3. Pharmacokinetic data requirements

A wide variety of pharmacokinetic data and analyses may be available for use in the BE derivation process. A fully developed PBPK model, while desirable, is not necessary for the process. While there is a preference for relying on human data to relate external dose to biomarker concentrations (Fig. 1, pathway 1), there was a recognition that when the exposure guidance value is based on animal data and there is information available on relevant internal dose metrics in the animals at the POD for the derivation (Fig. 1, pathway 2), this information could inform an internal dose-based derivation of a BE consistent with the exposure guidance value. This raises the issue of what uncertainty factors (UFs) are appropriate for use in conducting elements of an internal dose-based risk assessment (relationship 3, Fig. 1); no clear guidance has previously been developed for this issue. A more formal structure for the use of both animal and human data and application of appropriate UFs (discussed further below) was developed, including criteria for determining when pharmacokinetic data or models might replace pharmacokinetic components of default UFs. A key consideration is the degree to which there are available data on the active compound (parent or metabolite), mode of action, and critical dose metric; such data would help inform the use of animal and/or human data in derivation of a BE. An understanding of whether the animal metabolic pathways are similar to those in humans is also important. The closer the relationship between biomarker (e.g., blood concentration) and the critical effect (principal target organ effect), the more estimates of such concentrations in the critical animal study can be used with confidence. Conversely, the more removed the biomarker is from the dose metric and critical effect, the more modeling and human data are needed, with correspondingly less confidence in the BE. When animal pharmacokinetic data are used, the BE should be based on data derived in the species and preferably strain used in the study that served as the basis for the exposure guidance value.

Finally, for some data-poor compounds, information to support BE derivation could be developed relatively easily by conducting experiments to provide direct measurements of biomarker concentrations associated with the species, strain, and dosing regimen used in the critical study that underlies the exposure guidance value derivation. Some researchers have previously recognized this potential approach and have recommended that such data routinely be collected during key chronic toxicity studies during product development (NRC, 2006; Saghir et al., 2006; Bahadori et al., 2007; Barton et al., 2006). Under these conditions, a classical pharmacokinetic experiment or model is not strictly necessary for the development of a BE, since the information regarding biomarker concentration at the point of departure in the key animal study can be informative in the evaluation of measured biomarker concentrations in human populations (discussed further below).

3. Assessment of inter- and intra-species pharmacokinetic variability and default pharmacokinetic UF components

Risk assessments for non-cancer endpoints typically proceed from a POD to an exposure guidance value through the application of UFs that account for pharmacokinetic and pharmacodynamic variability both between animals and humans and among humans (Dorne et al., 2001). There is an important interplay between default pharmacokinetic (PK) UFs routinely used in the derivation of exposure guidance values and the incorporation of available pharmacokinetic data and models in the derivation of BE values. The BE relies on some measure of internal dose, which has the potential to reduce uncertainty in the risk assessment process (Andersen, 1987, 1995). As a result, the modeler could or should, in certain situations, replace default PK uncertainty factors with modeling approaches. The following sections capture some of the key considerations regarding UFs.

3.1. Inter-species pharmacokinetic variability

A central tenet in toxicology and pharmacology is that for an equivalent critical dose metric (the concentration of the active chemical moiety [parent or metabolite] at the critical organ or site of action of relevance to the toxic response of interest), most species will respond in an equivalent toxicological and pharmacological manner (Andersen, 1987, Andersen et al., 1995; Dorne et al., 2001). As a result, modern risk assessments attempt to identify the mode of action and associated critical dose metric as a component of risk assessment to inform inter-species extrapolations. In the ideal situation, the biomarker for a given chemical would be identical to the critical dose metric; thus, biomonitoring data would provide the most toxicologically relevant internal dose measure. However, the concentration of a biomarker in blood, urine, or other biological medium is not necessarily identical to the critical or appropriate dose metric consistent with the mode of action for toxicity underlying the derivation of a toxicity value.

The typical process for derivation of exposure guidance values incorporates a default inter-species UF component for pharmacokinetic differences ($UF_{A\text{-}PK}$) or allometric scaling (e.g., using a form of body weight scaling to account for relative surface area to bodyweight) to account for presumed inter-species differences in pharmacokinetics. When exposure guidance values have been derived

using internal dose as the metric for extrapolation, modeling has replaced the use of default inter-species PK UFs ($UF_{A\text{-}PK}$) (see USEPA, 1999). In deriving BEs, it is recognized that if the biomarker concentration is essentially identical to (or directly proportional to) the critical dose metric, then plausibly the $UF_{A\text{-}PK}$ could be replaced in the derivation of a BE value with the use of chemical-specific animal pharmacokinetic modeling or data to estimate the biomarker concentration at the POD in the critical study. However, if the biomarker concentration is not directly related to the critical dose metric, or if the relationship is dependent on external exposure patterns (for example, route of exposure or temporal variations in exposure pattern), then replacement of the $UF_{A\text{-}PK}$ with modeling or pharmacokinetic data would not necessarily be appropriate. Simulation studies for compounds with both animal and human pharmacokinetic models and a range of properties could illuminate the relationship between typical biomarkers (e.g., parent compound in blood) and various hypothetical critical dose metrics (e.g., AUC of parent or metabolite in a target organ, peak metabolic rate, etc.) under a variety of exposure scenarios and for a range of chemical properties.

An example in which the relationship between the critical dose metric and the biomarker concentration in blood could be uncertain or different under different exposure conditions is the case in which a metabolite produced in liver is responsible for toxicity. The estimated exposures by the inhalation and oral routes that produced the same amount of metabolite in liver would be associated with different blood levels of the parent compound (for example, see BE dossier for trihalomethanes in this issue). The differences would be larger for a compound with a high liver extraction (i.e., a large first pass effect for the oral route).

3.2. Intra-species pharmacokinetic variability

The typical process for derivation of exposure guidance values also incorporates a default intra-species uncertainty factor component ($UF_{H\text{-}PK}$) to account for variability in pharmacokinetics between typical and pharmacokinetically sensitive members of the population. As with the inter-species pharmacokinetic UF component, under some conditions (notably, where the biomarker concentration is directly related to the critical dose metric) a human pharmacokinetic model could replace application of the $UF_{H\text{-}PK}$ in extrapolation from the BE_{POD}. However, in this case, the human "model" is directly represented by biomonitoring data. For example, in the case where the parent compound is the active agent and metabolism results in detoxification and elimination, pharmacokinetically sensitive individuals would be those with slower metabolism. These individuals would manifest higher concentrations of parent compound in blood for the same external exposure, and if this is the biomarker being measured, the pharmacokinetic sensitivity would be reflected in the elevated blood concentrations measured. Thus, in the case where the critical dose metric is well-approximated by the biomarker, the appropriate BE value can be derived from the BE_{POD} and identification of a target margin of exposure which accounts for pharmacodynamic factors (inter- and intra-species) as well as appropriate inter-species pharmacokinetic extrapolation (as discussed above), but which does not include the default $UF_{H\text{-}PK}$ component. However, when the relationship between the biomarker concentration and critical dose metric is not well-understood or is indirect, a default or chemical-specific model-derived $UF_{H\text{-}PK}$ can be applied. The decision process for this determination is discussed in the next section of this Workshop report.

Human pharmacokinetic data and PBPK models (where available) can help illuminate the degree of interindividual variability predicted under a variety of exposure. Such information may be informative in the interpretation of biomarker concentrations, and should be included in the derivation documentation if available.

4. Flowchart and process for BE derivation

There are two basic elements of the derivation process illustrated in Fig. 3:

- Identification of the biomarker concentration at the human equivalent POD (BE_{POD}); and
- Identification of a target margin of exposure (MOE) to be applied to the BE_{POD} to derive the BE value commensurate with the exposure guidance value.

The target MOE is influenced by whether the POD was derived from an animal or human study, the degree of confidence in the relationship between the biomarker and the critical dose metric, and whether animal or human pharmacokinetic data (or both) are available. The following sections discuss the derivation of the BE_{POD} and the identification of appropriate target MOEs under various conditions.

4.1. Derivation of BE_{POD}

The POD that underlies an exposure guidance value is the recommended starting point for deriving BE values. The advantages of this approach include:

- Increased transparency in the derivation;
- Ability to provide additional perspective for interpretation of human biomonitoring data;
- Potential for harmonization between exposure guidance value derivations from different agencies (or to clarify potential sources of differences);
- Potential to harmonize the approaches to derivation of BEs for exposure guidance values based on cancer versus non-cancer endpoints;
- Ability to evaluate potential replacement of selected default uncertainty factors with chemical-specific pharmacokinetic data or modeling (discussed further below); and
- Ability to leverage information on mode of action and relevant internal dose metrics when available.

Exposure guidance values derived by different organizations use a variety of starting points including the no observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL), or benchmark dose from human or animal studies. Therefore, a flexible decision-tree process is required to derive BEs from these values (Fig. 3). In the BE derivation process, the POD is defined as a duration-adjusted and LOAEL-to-NOAEL-adjusted external dose or exposure concentration, that is, an exposure level equivalent to a NOAEL or appropriate benchmark dose or its lower bound. Two types of duration adjustment are frequently used in deriving exposure guidance values and would therefore be used in BE derivation. One adjustment commonly applied extrapolates from discontinuous to continuous exposures (e.g., 5 days per week to 7 days per week). The second adjustment relates to the overall duration of the exposure and the anticipated toxicities, for example acute or chronic effects. Chronic exposure guidance values are often derived using an adjustment from studies with shorter exposure periods, notably subchronic.

Once appropriate conversions for LOAEL to NOAEL and duration adjustment have been applied to arrive at the POD for BE derivation, the inter-species extrapolation process begins (if the exposure guidance value is based on an animal study). The POD from the ani-

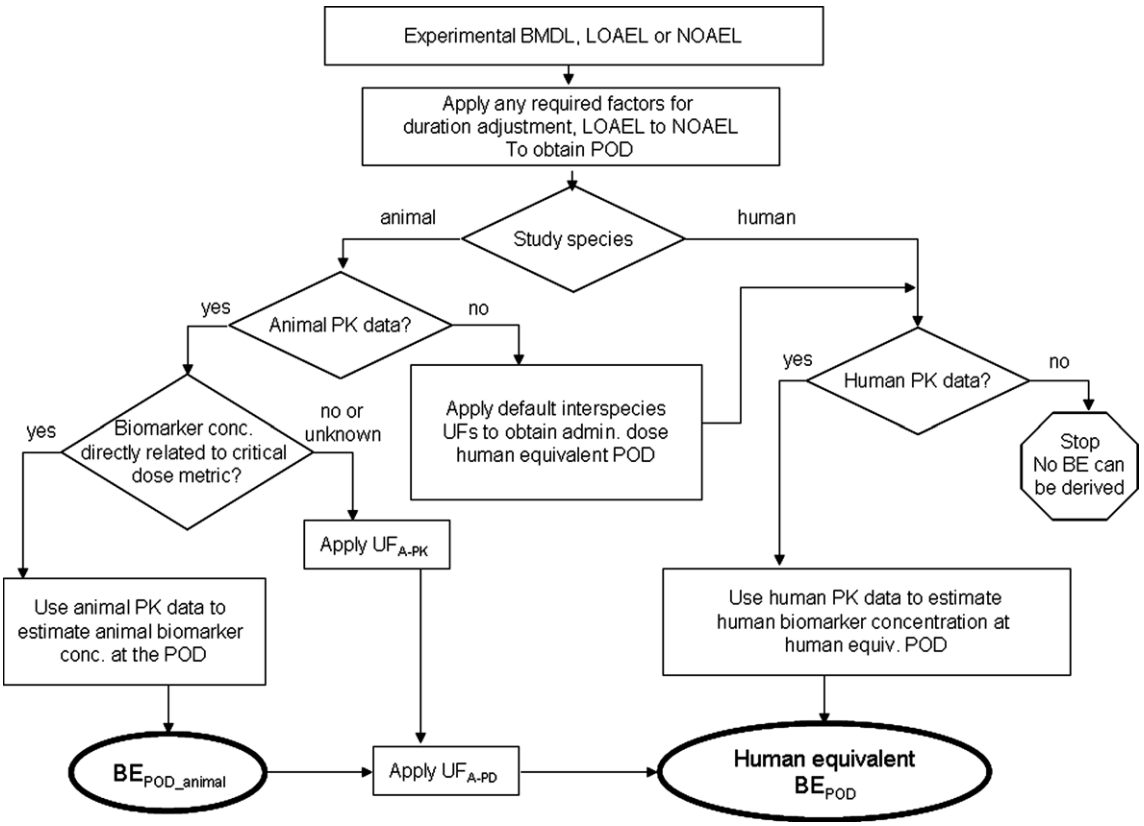

Fig. 3. Flowchart of the process for derivation of BE_{POD} values under combinations of animal and human toxicity data and either animal or human pharmacokinetic data or models. Key steps in the derivation include the evaluation of the application of default uncertainty factor components based on understanding regarding the relationship between the biomarker and the critical or relevant dose metric. BMDL, lower bound on the benchmark dose; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; POD, point of departure; $UF_{A\text{-}PK}$, pharmacokinetic component of the default inter-species uncertainty factor; $UF_{A\text{-}PD}$, pharmacodynamic component of the default inter-species uncertainty factor.

mal study will be converted to a human equivalent POD through application of appropriate modeling (on an internal dose basis when data or models permit) or default inter-species UFs (on an external dose basis), or some combination of the two approaches depending upon the available data. All such conversions and applications of modeling or inter-species UFs should be clearly described in the BE documentation along with the rationale for choosing among various alternatives. Fig. 3 presents a flowchart that captures the rationale behind the derivation of BE_{POD} values based on data from either animal or human studies under conditions in which *either* animal or human pharmacokinetic data (but not both) are available. Two key estimates of BEs are possible: the BE_{POD_animal} and the human equivalent BE_{POD}. The BE_{POD_animal} reflects the biomarker concentration in the animal species of interest at the POD (after adjustment for duration, etc., as discussed above). The human equivalent BE_{POD} reflects biomarker concentrations consistent with the POD following application of appropriate inter-species extrapolation approaches (via application of pharmacokinetic data or modeling and/or default uncertainty factor components, as appropriate). In cases in which the exposure guidance value is based on human health effect data rather than on an animal study, or in cases in which no animal pharmacokinetic data are available, no BE_{POD_animal} will be derived.

For data-rich compounds, pharmacokinetic data or models may be available for both animals and humans. Fig. 4 presents approaches that could be used in the situation in which the exposure guidance value is based upon an animal toxicity study but pharmacokinetic data are available for both animals and humans (if the exposure guidance value is based on human toxicity data, animal pharmacokinetic data are irrelevant). Two pathways are possible, leading to a BE_{POD_animal} and subsequently to a human equivalent BE_{POD}; the different pathways are distinguished by whether or not the critical or highly relevant dose metric is known for the critical toxicity endpoint. The resulting human equivalent BE_{POD} value can be combined with the appropriate target minimal margin of exposure (MOEs) (identified in Table 1 and discussed in the next section) to derive the target BE value.

4.2. UFs/target MOEs: non-cancer and non-linear cancer assessments

Risk assessments for non-cancer endpoints typically proceed from a POD to an exposure guidance value through the application of UFs that account for pharmacokinetic and pharmacodynamic variability both between animals and humans and among humans (Dorne et al., 2001). In addition, some non-linear cancer risk assessments rely upon a similar approach, with identification of a POD for a key event in the mode of action and application of UFs. The decision points for application of inter-species UFs are presented in Figs. 3 and 4. Table 1 presents a breakout of the default intra-species UFs with an assessment of the appropriateness of the application of these factors to the human equivalent BE_{POD} value resulting from the process detailed in Figs. 3 and 4.

In terms of the application of default uncertainty factors to derive BE values from exposure guidance values, the pharmacokinetic components of both the inter- and intra-species default UFs can appropriately be replaced with modeling approaches under some circumstances. In the case of the inter-species pharmacokinetic component ($UF_{A\text{-}PK}$), use of an internal dose metric based on mod-

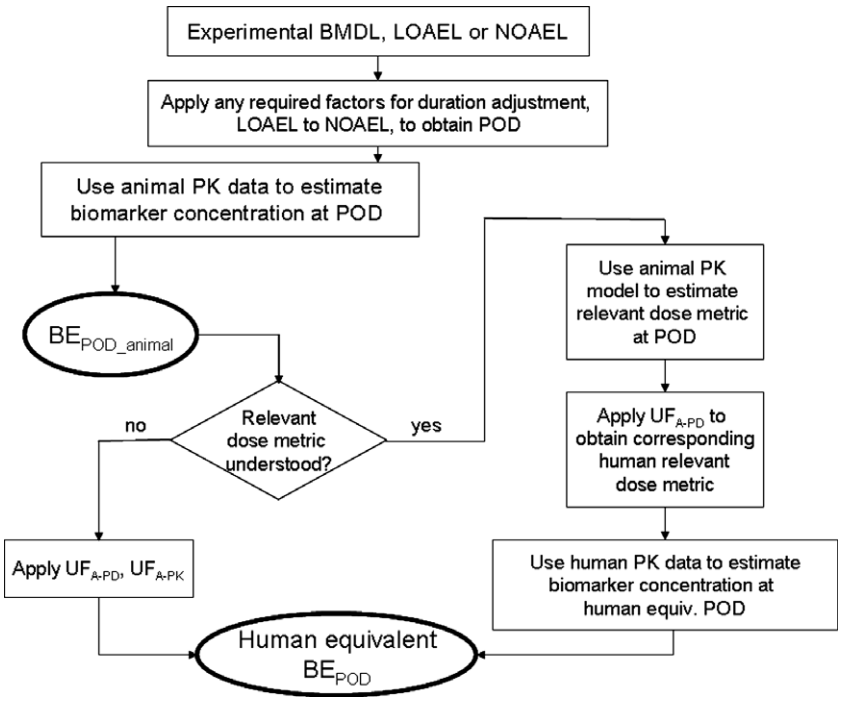

Fig. 4. Flowchart of BE_{POD} derivation when the exposure guidance value is based on data from an animal toxicity study and both animal and human PK data are available. The resulting human equivalent BE_{POD} values can be used with appropriate UFs identified from Table 1 to derive the target BE values. Abbreviations as in Fig. 3.

Table 1
Assessment of chemical-specific target minimal margin of exposure (MOE) (or composite uncertainty factors) between the estimated biomarker concentration at the human equivalent POD (human equivalent BE_{POD}; see Figs. 3 and 4 for derivation flowcharts) and measured biomarker concentrations in the general population for reference exposure values based on non-cancer endpoints

UF component[a]	Biomarker is directly related to critical target tissue dose metric	Biomarker is distant from critical target tissue dose metric or relationship is unknown
$UF_{H\text{-}PD}$	Yes	Yes
$UF_{H\text{-}PK}$	No	Evaluate[b]
Additional UFs[c]	As specified by deriving agency	
Composite UF	Product of component UFs	

The target BE value commensurate with the exposure guidance value for screening general population biomonitoring data is derived by dividing the human equivalent BE_{POD} by the appropriate composite UF. *Note:* Inter-species UFs (pharmacokinetic and pharmacodynamic) are accounted for in the derivation of the human equivalent BE_{POD}).

[a] For the purposes of this discussion, the value of each UF component is assumed to be one-half an order of magnitude, rounded to 3. Other apportioning of these UF components could be contemplated.

[b] As discussed in text, $UF_{H\text{-}PK}$ may be appropriate if the potential for intra-species variations in the relationship between the biomarker concentration and the critical dose metric exist, and this factor should be evaluated on a case-by-case basis.

[c] May be applied for database uncertainties or other reasons, depending on regulatory agency determinations.

eling for extrapolation between animals and humans replaces this component when the biomarker is judged to be directly related to the critical internal dose (see Fig. 3). However, if the relationship between the biomarker and the critical dose metric is not well-understood, or if that relationship may be different in humans than in the laboratory animal species, this component of the default uncertainty factor may be retained.

The relationship between measured biomarker and critical internal dose also influences the decision on use of the pharmacokinetic component of the default intra-species uncertainty factor ($UF_{H\text{-}PK}$). If the biomarker concentration is expected to be directly proportional to the critical internal dose, no $UF_{H\text{-}PK}$ factor is necessary because the measurement of biomarker concentration in humans explicitly addresses "pharmacokinetic sensitivity": persons who are pharmacokinetically sensitive will develop higher biomarker concentrations than those who are average for the same external exposure. However, when the relationship between the biomarker and the critical internal dose is less certain, the possibility exists that individuals within the population with the same measured biomarker concentration may develop different concentrations of the critical internal dose. In such a case, an evaluation should be made as to whether the $UF_{H\text{-}PK}$ should be retained (see Table 1).

The determination regarding whether each of these pharmacokinetic components of default uncertainty factors has been accounted for through modeling or the measurement of biomarkers in humans is affected most strongly by the confidence in the understanding of the relationship between the measured biomarker and the critical target tissue dose metric, and by understanding regarding potential inter- or intra-species variations in that relationship. The evaluation of the relationship between the biomarker and a relevant or critical internal dose should be based on consideration of several factors. For example, is the biomarker upstream or downstream of the likely active agent in the metabolic pathway of the compound? If the active agent is likely to be the parent compound or a major metabolite of that compound, analysis of the parent compound in blood will likely provide a good surrogate for the relevant or critical dose metric. However, if the likely active agent is a minor metabolite or a metabolite that results from saturation of a major pathway (e.g., only becomes prominent when glutathione is depleted), then parent compound in blood may not be directly related to the critical or relevant dose metric. Similarly, if the biomarker is downstream of the active agent in the metabolic pathway, or on a parallel metabolic pathway that is not directly related to toxicity, the biomarker may not be directly related to the critical dose metric. Finally, if the biomarker is a urinary excretion product, and the concentration of the biomarker in urine is not directly related to toxicity, a BE based on such urinary biomarkers is probably most accurately reflective as a biomarker of exposure,

and the full suite of UFs applied in derivation of the external exposure guidance value (including pharmacokinetic components) should be applied.

The application of a pharmacokinetic component of either the inter- or intra-species uncertainty factor in situations where the relationship between the biomarker and critical dose metric is either indirect or unknown is analogous to, but not identical to, the application of the pharmacokinetic components in external dose-based risk assessments. In risk assessments based on external doses, the pharmacokinetic components represent uncertainty regarding the difference between species (or between individuals) in the critical internal dose resulting from a given external dose or exposure. The traditional use of a value of approximately one-half an order of magnitude for this factor is not directly based in any empirical science, although it is somewhat consistent with allometric extrapolations based on surface area or bodyweight scaling (Andersen et al., 1995; Dorne et al., 2001). Use of the same pharmacokinetic components to represent uncertainty regarding the relationship between the measured biomarker and the critical dose metric of interest is intellectually consistent with the external application of these components. However, application of the same pharmacokinetic components might be expected to be more conservative in the case of the application to biomarkers, because such biomarkers would be expected to be more informative of the critical dose metric than external exposures. The relationship between various biomarkers and theoretically plausible critical dose metrics (and inter- and intra-species considerations in evaluation of this relationship) is an area that might be explored through simulation exercises with existing PBPK models for a variety of classes of compounds.

Conventional risk assessments based on external dose typically aim for composite MOEs between animal NOAELs and human exposures of at least 100 (accounting for a factor of 10 for inter-species extrapolation and 10 for within human variability). In contrast, the corresponding target MOEs based on internal dose assessments and biomonitoring data may be 10 (accounting for a factor of 3 for inter-species extrapolation of pharmacodynamic differences and a factor of 3 for within human variability in pharmacodynamics) or less if the exposure guidance value is based on human toxicology data or alternative UFs are used in the development of the exposure guidance values (Dorne and Renwick, 2005; IPCS, 2005). This can occur when the pharmacokinetic components of the typical inter- and intra-species uncertainty factors are replaced by modeling and biomonitoring data, respectively.

The human equivalent BE_{POD} and the BE value commensurate with the exposure guidance value provide a basis for demarcating biomarker concentrations that represent low, medium, and high priority for risk assessment follow-up. Fig. 5 illustrates the presentation of these values, as well as the BE_{POD_animal}, for a hypothetical compound along with the designation of ranges of low, medium, and high priority for risk assessment follow-up. The meaning of these terms and the implications for interpretation of both population and individual biomonitoring data are discussed in more detail in the accompanying paper on BE communications.

4.3. Target MOEs: linear cancer assessments

Current cancer risk assessments based on animal data for chemicals using a linear no-threshold assumption are conducted through a four step process:

- Selection of a tumor endpoint or endpoints from an animal study;
- Identification of an external dose point of departure through benchmark dose modeling (usually the LED_{10});

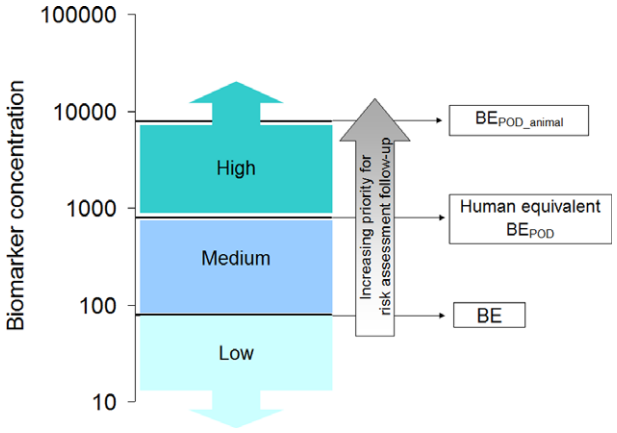

Fig. 5. Presentation of a hypothetical BE with the human equivalent BE_{POD} shown as the demarcation between regions of medium and high priority for risk assessment follow-up and the BE as the demarcation between regions of low and medium priority for risk assessment follow-up.

- Conversion of the external dose LED_{10} to a human equivalent LED_{10}; and
- Linear extrapolation of the human equivalent LED_{10} to risk-specific doses at selected target cancer risk levels.

Typical target cancer risk levels of interest are in the range of 10^{-6}–10^{-4} risks. These correspond to MOEs from the LED_{10} of 100,000–1000, respectively.

In this framework, the animal exposure dose or concentration is converted to the human equivalent using a number of methods including allometric scaling (for example, by adjusting applied doses by body weight raised to a power of 2/3 or 3/4 for oral doses, essentially replacing the $UF_{A\text{-}PK}$ typically applied in the non-cancer framework, or application of adjustments for inhaled concentrations consistent with the USEPA RfC methodology [USEPA, 2002]). No additional UF components, either inter- or intra-species, are explicitly included in the cancer risk assessment process.

Fig. 6 illustrates the process for the estimation of BE values associated with cancer risk levels under a linear extrapolation assumption. This framework addresses the derivation of a BE_{POD} based on animal bioassay data in combination with either animal pharmacokinetic data (the BE_{POD_animal}) or human pharmacokinetic data (human equivalent BE_{POD}. Whichever BE_{POD} value is derived through this process can then be linearly extrapolated to identify risk-specific biomarker concentrations at target risk levels of interest.

5. Framework for presentation of BE values and documentation of confidence

Transparency in the presentation and documentation of confidence in the BE derivation is an important goal. The Panel discussed at some length the challenges in presenting the results of the BE derivation process, addressing issues such as nomenclature, method of presentation, and methods of discussing the meaning of measured biomarker concentrations in excess of identified BE values. Many of these issues are discussed at greater length in the Workshop report on communication that is included in this volume (LaKind et al., 2008).

Use of subscripts or other notations to the term "BE" could be confusing and should be avoided when communicating to the lay public, but may be warranted and necessary when communicating to health risk assessment communities and in the context of the derivation documentation. Thus, no subscripts to designate biological matrix (e.g., blood or urine) or the underlying exposure guid-

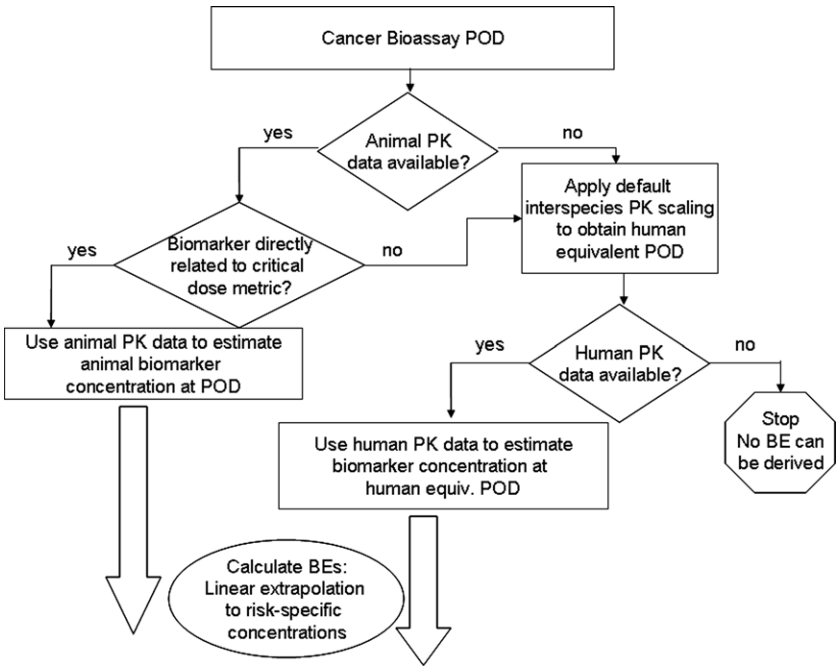

Fig. 6. Flowchart of the derivation of BEs associated with specific cancer risk levels under the assumption of a linear cancer risk assessment model depending upon availability of animal and human PK data and relationship between biomarker and critical dose metric.

ance value (e.g., RfD or MRL) are used in the designation of BE values. However, the term "BE_{POD}" to designate the estimated biomarker concentration associated with the point of departure underlying the exposure guidance value is used. A table with columns can present the specific information that might otherwise be designated through the use of subscripts. In addition, where there is uncertainty in the estimates of biomarker concentration associated with POD due to model or data uncertainties, the range of estimated values based on available data can be presented. Table 2 is an example of the presentation of BE values for a hypothetical compound (additional examples are provided in the accompanying BE dossiers).

Table 2 also contains a column for presentation of an overall confidence rating. Two major areas are important in evaluating the confidence in the BE values:

- Understanding of and confidence in the methods used (the kinetic data and/or model) to convert external exposure to the estimated internal biomarker concentration. This includes consideration of:
  - whether human kinetic data are available;
  - the degree of extrapolation required from the range of observed kinetic data; and
  - the possibility of saturation or non-linear kinetics in the dose range(s) of interest in the exposure guidance value derivation.
- Understanding of and confidence in the relationship between the biomarker concentration and the biological response that serves as the basis of the exposure guidance value derivation. This includes evaluation of:
  - the understanding of the critical dose metric (including mode of action information);
  - whether the biomarker is on the metabolic pathway resulting in toxicity or on a parallel pathway, and whether the biomarker is upstream or downstream of the presumed toxic moiety; and
  - the likelihood of substantial inter- or intra-species differences in the relationship between the biomarker concentration and the critical dose metric.

In most cases detailed information will not be available on all of these factors. However, exposure guidance values are generally defined without complete understanding of these and other relevant factors, and uncertainties in these areas do not preclude the development and use of exposure guidance values, and should not preclude the development and use of BE values as long as the discussion of confidence is transparent on these and any other relevant issues identified on a chemical-specific basis. The composite confidence rating can be assigned based on the assessment of each of the factors presented above.

Table 2
Example summary table for presentation of BE values

Underlying exposure guidance value	Analyte	Biological matrix	Human equivalent BE_{POD}	Target BE	Confidence
USEPA RfD	Parent	Blood	120 ng/mL	40 ng/mL	High[a]
	Metabolite	Urine	30–60 μg/g creatinine	3–6 μg/g creatinine	Medium[a]

The underlying exposure guidance values, and the methods used to estimate the BE values, would be described in more detail in accompanying text and table(s).
[a] A summary of the considerations leading to the confidence rating can be presented here.

6. Specific technical issues and considerations

Individual chemical characteristics lead to a variety of technical issues in BE development that can be informed by the general BE approach developed above, but which entail additional considerations. Such issues include BE development for transient compounds, addressing variability in urinary biomarker concentrations due to hydration status, the use of non-specific metabolites as biomarkers, and the interpretation of biomonitoring data for longer-lived compounds.

6.1. Transient compounds

For short-lived compounds, the profiles of the chemicals in blood and/or urine may be transient, with short-term peaks following daily oral exposures. Likewise, in the animal studies (and sometimes human studies) that underlie the development of the exposure guidance value, the dosing regimen (or exposure for humans) may have resulted in transient peak chemical concentrations in the animals or humans, and those peaks may have relevance for the resulting health endpoint of interest. Measurement of biomarker concentrations of such compounds at any time point provides little information regarding past biomarker concentrations, and the biomarker concentrations are highly sensitive to exposure route, scenario, or sampling time in relation to exposure, among other considerations (NRC, 2006; ECETOC, 2005).

While transient, rapidly eliminated compounds are not ideal candidates for BE development due to the issues discussed above, information regarding pharmacokinetics of these compounds can still be used to develop some guidelines for interpretation of biomonitoring data for these compounds, with appropriate cautions and qualifying information. In particular, estimates of mean or time-weighted average biomarker concentrations that would be consistent with the POD or exposure guidance value could be derived. Such values could be used for comparison to the population average biomarker concentrations in a given biomonitoring study. However, the interpretation of short-lived compounds using the BE approach should also include:

- When possible, estimates of plausible peak levels of biomarkers associated with the PODs or exposure guidance values;
- Communication materials that include a discussion of the likelihood of peaks that substantially exceed average biomarker concentrations (potentially by several-fold to an order of magnitude, depending upon the exposure scenario and the half-life of the compound); and
- A clear and prominent acknowledgment that BE values for such compounds are of limited value for the interpretation of isolated biomarker measurements in individuals.

For such compounds, cross-sectional biomonitoring data alone are likely to be limited for characterizing exposure and risk, and additional data collection may be needed to effectively interpret biomonitoring data. Such data could include multiple, serial sampling of biomarkers in individuals, information on time since the last likely exposure collected in conjunction with the biomonitoring sample, or information on external exposure. In some cases, alternative biomarkers (for example, specific urinary metabolites) that are more persistent might be identified and provide a more reliable estimate of integrated, but not peak, exposures.

6.2. Long-lived compounds

The interpretation of the critical toxicological or epidemiological studies in terms of dosimetry depends, in part, on the frequency of exposure as compared to how rapidly the compound is eliminated. When the rate of elimination is relatively slow compared with the frequency of exposure, the chemical is likely to build up to approximate steady state levels with larger or smaller oscillations in the peak or maximum levels and the trough or minimum levels around the steady state concentration. When the rate of elimination is fast compared to the frequency of exposure, there will tend to be large periodic oscillations in the levels of chemical. Thus, "longer-lived" compounds (those that have a longer biological half-life) would likely build to near steady state conditions such that fluctuations in exposure regimen (once versus several times per day, for example) have little impact on the measured biomarker concentrations. Such compounds and biomarkers have been the focus of substantial biomonitoring efforts in the past (for example, lead, cadmium, some persistent organochlorine compounds, and hemoglobin adducts of a variety of compounds). The time to reach approximate steady state in the body is determined by the slowest kinetic process involved in distribution and elimination of the compound; in practice, for biologically persistent compounds, this is the rate of elimination.

The ultimate determinant of the biologically effective dose (or the appropriate dose metric) is the toxicity process (mode of action) leading to the effect. However, understanding whether or not the exposures would have been at steady state for a substantial period of the critical study can be valuable. Approximately steady state levels are achieved in 3–5 half-lives. For chemicals with a whole body half-life of a week or longer, a 28-day toxicity study would have generally increasing levels of the chemical throughout while a 90-day toxicity study would approximate steady state for at least two months. At or near steady state, several dose metrics are all highly correlated so the choice of the area under the concentration curve, the maximum, average, and minimum concentrations, or other measures may all give fairly similar results. Prior to achieving near steady state conditions, different dose metrics may provide fairly different dose–response results (e.g., AUC on the first day, average daily AUC during the study, versus AUC on the last day). This is particularly the case for developmental effects where the critical window may be fairly brief, but its timing unknown.

The process of estimating BEs will need to include consideration of whether the critical study was largely under pre-steady state or near steady state conditions and the uncertainties in dose metric selection will be greater for studies largely representing pre-steady state conditions. In addition, for chemicals with long human half-lives, it is possible that levels could change with age reflecting both accumulation and changes in exposure. These changes may need to be addressed along with issues related to intra-species variation, as they contribute to variability observed in biomarker concentrations.

6.3. Non-specific metabolites

Many biomonitoring studies have measured the concentration of chemicals in blood or urine that are non-specific metabolites or degradation products of a variety of parent compounds. Except in the case where the non-specific metabolite is the toxic moiety of interest for multiple compounds, such biomonitoring data present additional challenges under any interpretation framework, including the BE framework. Non-specific metabolites may arise from parent compounds that have the same mode of action but substantially different relative potencies (see, for example, organophosphate compounds); from parent compounds with different modes of action (for example, 1-napthol, which can arise from both carbaryl and from naphthalene), and may also be present in the environment and thus encountered directly, as well as occurring as the metabolite of another compound. Except for the case where

the measured chemical is also the active agent, the uncertainties will be larger when using non-specific biomarkers.

Factors including analytical detection or method issues, cost of analysis, and comparison to historical data have created interest in the interpretation of non-specific markers. It is also possible that methods for establishing BEs for non-specific biomarkers may facilitate the interpretation and influence the design of future cumulative biomarkers of exposure and effect, such as gene expression or endogenous protein patterns.

In general, the supporting research to develop BEs for non-specific biomarkers has not been completed. It may not be necessary to identify the levels of all chemicals contributing to a common biomarker, but estimating dominant agents or probable relative contributions for the population may be sufficient. A possibility is to validate the relationships between available information such as market surveys or available environmental monitoring data and ratios of chemical-specific biomarkers for compounds that also contribute to a common biomarker of interest. Other strategies may be adopted from occupational monitoring settings or controlled laboratory studies that produce methods to differentiate a particular chemical from background levels for the common biomarker. Although these activities are not in the scope of the derivation of BEs, the BE framework provides guidance and justification for such research, as well as impetus to develop analytical capability for determination of more specific markers of exposure.

6.4. Urinary markers—variability due to hydration status

Measurement of chemical exposure is often conducted via analysis of a biomarker (parent chemical or metabolite) in non-invasive urine samples. These urine samples can be spot samples, timed void samples, or composite 24-h collections. Several groups have evaluated the impact of sample collection interval on the accuracy of biomarker concentration determination. Kissel et al. (2005) have shown that biomarker and creatinine concentrations may vary widely in four, separate timed urine voids. The most representative time interval identified was the first morning void (FMV). However, even the FMV sample can deviate substantially from true daily exposure, as Scher et al. (2007) found that concentrations of 2,4-D and 3,5,6-trichloropyridinol (TCPy) in FMV samples varied 2- to 3-fold from 24-h composite values.

These variations in timed sample biomarker concentrations may be a result of inconsistent exposures, short to moderate half-life kinetics and/or variations in urine production rates (especially in occupational settings). To compensate for within-day variations in urinary volume, researchers often normalize biomarker levels to urinary creatinine, average daily urine volumes, or specific gravity, and adjustments may be gender-specific. Corrections by average daily urine volume or creatinine excretion are the most common adjustment methods, however both of these parameters can exhibit significant inter-day and interindividual variation (2- to 4-fold or more in adults Boeniger et al., 1993; Ballauff et al., 1988; Bingham et al., 1988; Newman et al., 2000; Remer et al., 2002, 2006). The concentration of creatinine in spot urine samples collected in children can vary up to 70-fold (Kissel et al., 2005); however, variation in daily (24-h composite) excretion of creatinine in children is not as great (2- to 4-fold) and is similar to the variation observed in adults (Remer et al., 2002). The optimum adjustment method must be compound-dependent, based on the mechanism of renal clearance, and should be determined for each biomarker studied. Ideally, this adjustment evaluation can be conducted during any initial controlled exposure scenario, in an occupational setting or as part of an approved study of the absorption, distribution, metabolism, and elimination for a compound.

The case studies for 2,4-D and acrylamide (Hays and Aylward, 2008) utilize different approaches for creatinine correction of biomarker levels. Creatinine-corrected 2,4-D urine levels for adults were calculated by Monte Carlo-generated creatinine concentrations based on variations in age, height and weight. Data for children's 2,4-D levels were calculated arithmetically from empirical data of Remer et al. (2002). Creatinine-corrected acrylamide metabolite *N*-acetyl-*S*-(2-carbamoylethyl)cysteine (AAMA) levels were calculated arithmetically from empirical data from several sources. The use of Monte Carlo simulation for estimation of creatinine levels in human volunteers is an excellent means of capturing inter-individual variation in BE calculations, and therefore replacing the need for inter-individual uncertainty factors. The Monte Carlo analysis showed that because both total mass of exposure at an exposure guidance value and creatinine production are related to bodyweight, the overall impact of creatinine production variability on predicted biomarker concentrations at the exposure guidance value was relatively small. Therefore, point estimates of estimated creatinine-corrected urinary concentrations can be used in BE derivations. These calculations do not account for other sources of variability including inter-individual differences in metabolic rate.

7. Conclusions

There are several over-arching conclusions regarding the guidelines for BE derivation and application:

- The derivation of BE values provides a screening tool that can assist in interpretation of human biomonitoring data within the context of the existing risk assessment paradigm, and may provide additional insights beyond those afforded by the conventional external dose-based risk assessment approach.
- The consideration of internal dose metrics in the derivation of BE values allows consideration of replacement of default UF components with modeling or pharmacokinetic data on a chemical-specific basis.
- The degree to which default UFs may be replaced with pharmacokinetic modeling depends on how well the chemical-specific mechanism of action is known, how closely related the biomarker is to the critical dose metric, and the availability of human and/or animal pharmacokinetic data relating the critical dose metric and the biomarker (when they are different).
- Like the exposure guidance values from which BEs are derived, BEs do not provide insights into the possibility of health effects occurring in human populations. Rather, BEs provide screening tools for risk management purposes by placing biomonitoring levels into context with existing exposure guidance values derived to protect public health.
- BEs derived for both the exposure guidance values and underlying PODs provide valuable insights for interpretation of biomonitoring data in a risk management context.
  - BEs (associated with the exposure guidance value) represent levels of biomarkers that pose low priority for risk assessment follow-up. Thus, when levels of biomarkers among a population are below the BE, this warrants a low priority for risk assessment follow-up. In the context of interpreting biomonitoring data, risk assessment follow-up includes: careful evaluation of the validity and confidence in the exposure guidance value(s), consideration of conducting exposure assessments to better understand routes and sources of exposure, and possibly implementation of risk management practices to reduce exposure(s) if warranted.
  - Biomarker concentrations exceeding the human equivalent BE_{POD} indicate increasing priority for risk assessment follow-up.

- Transparency in BE derivation is important.
- Confidence in BEs will be variable, and should be conveyed in a transparent fashion.

The flowcharts presented in Figs. 3, 4 and 6 and the considerations in selection of target MOEs in Table 1 represent the BE Model. This approach should be used to guide the derivation of BEs. As with any scientific guidance, variations in details and approaches will likely arise frequently in consideration of specific chemicals. In such situations, it is important to adhere to the spirit of these guidelines and explain the basis for alternative decisions or choices. As with any scientific guidance, as these BE derivation guidelines are put to practice, there will be lessons learned as new BEs are derived.

Disclaimer

This work was reviewed by EPA and approved for publication, but does not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

Conflict of interest disclosure statement

The authors declare that they have no conflicts of interest.

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References

Andersen, M.E., 1987. Tissue dosimetry in risk assessment, or what's the problem here anyway? Drinking Water Health 8, 8–23.

Andersen, M.E., 1995. What do we mean by. . .dose? Inhal. Toxicol. 7, 909–915.

Andersen, M.E., Clewell III, H., Krishnan, K., 1995. Tissue dosimetry, pharmacokinetic modeling, and interspecies scaling factors. Risk Anal. 15 (4), 533–537.

Aylward, L.L., Hays, S.M., 2008. Biomonotoring Equivalents (BEs) dossier for toluene (CAS No. 108-88-3). Regul. Toxicol. Pharmacol. 51, S27–S36.

Bahadori, T., Phillips, R.D., Money, C.D., Quackenboss, J.J., Clewell, H.J., Bus, J.S., Robison, S.H., Humphris, C.J., Parekh, A.A., Osborn, K., Kauffman, R.M., 2007. Making sense of human biomonitoring data: findings and recommendations of a workshop. J. Expo. Sci. Environ. Epidemiol. 17 (4), 308–313.

Ballauff, A., Kersting, M., Manz, F., 1988. Do children have an adequate fluid intake? Water balance studies carried out at home. Ann. Nutr. Metab. 32 (5–6), 332–339.

Barton, H.A., Pastoor, T.P., Baetcke, K., Chambers, J.E., Diliberto, J., Doerrer, N.G., Driver, J.H., Hastings, C.E., Iyengar, S., Krieger, R., Stahl, B., Timchalk, C., 2006. The acquisition and application of absorption, distribution, metabolism, and excretion (ADME) data in agricultural chemical safety assessments. Crit. Rev. Toxicol. 36 (1), 9–35.

Bingham, S.A., Williams, R., Cole, T.J., Price, C.P., Cummings, J.H., 1988. Reference values for analytes of 24-h urine collections known to be complete. Ann. Clin. Biochem. 25 (Part 6), 610–619.

Boeniger, M.F., Lowry, L.K., 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.

Dorne, J.L.C.M., Walton, K., Renwick, A.G., 2001. Uncertainty factors for chemical risk assessment: human variability in the toxicokinetics of CYP1A2 probe substrates. Food Chem. Toxicol. 39, 681–696.

Dorne, J.L., Renwick, A.G., 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. Toxicol. Sci. 86 (1), 20–26.

ECETOC, 2005. Guidance for the Interpretation of Biomonitoring Data, ECETOX Dox. No. 44, Brussels.

Fiserova-Bergerova, V., 1987. Development of biological exposure indices (BEIs) and their implementation. Appl. Ind. Hyg. 2, 87–92.

Fiserova-Bergerova, V., 1990. Application of toxicokinetic models to establish biological exposure indicators. Ann. Occup. Hyg. 34, 639–651.

Hays, S.M., Aylward, L.L., 2008. Biomonitoring Equivalents (BEs dossier) for acrylamid (AA) (CAS No. 79-06-1). Regul. Toxicol. Pharmacol. 51, S57–S67.

Hays, S.M., Becker, R.A., Leung, H.W., Aylward, L.L., Pyatt, D.W., 2007. Biomonitoring equivalents: a screening approach for interpreting biomonitoring results from a public health risk perspective. Regul. Toxicol. Pharmacol. 47 (1), 96–109.

IPCS (International Programme on Chemical Safety), 2005. Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration–response assessment. World Health Organization, Geneva. Available from: http://www.inchem.org/documents/harmproj/harmproj/harmproj2.pdf.

Kissel, J.C., Curl, C.L., Kedan, G., Lu, C., Griffith, W., Barr, D.B., Needham, L.L., Fenske, R.A., 2005. Comparison of organophosphorus pesticide metabolite levels in single and multiple daily urine samples collected from preschool children in Washington state. J. Expo. Anal. Environ. Epidemiol. 15, 164–171.

Kroes, R., Renwick, A.G., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schiltter, B., Schlatter, J., van Schothorst, F., Vos, J.G., Wurtzen, G., 2004. Structure based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. Foot Chem. Toxicol. 42, 65–83.

LaKind, J.S., Aylward, L.L., Brunk, C., DiZio, S., Dourson, M., Goldstien, D.A., Kilpatrick, M.E., Krewski, D., Bartels, M.J., Barton, H.A., Boogaard, P.J., Lipscomb, J., Krishnan, K., Nordberg, M., Okino, M., Tan, Y.-M., Viau, C., Yager, J.W., Hays, S.M., 2008. Guidelines for the communication of Biomonitoring Eqivalents: Report from the Biomonitoring Equivalents Expert Workshop. Regul. Toxicol. Pharmacol. 51, S16–S26.

Morgan, M.S., 1997. The biological exposure indices: a key component in protecting workers from toxic chemicals. Environ. Health Perspect. 105, 105–115.

National Research Council (NRC), 2006. Human Biomonitoring for Environmental Chemicals. National Academies Press, Washington, DC.

Newman, D.J., Pugia, M.J., Lott, J.A., Wallace, J.F., Hiar, A.M., 2000. Urinary protein and albumin excretion corrected by creatinine and specific gravity. Clin. Chim. Acta 294, 139–155.

Rappaport, S.M., Symanski, E., Yager, J.W., Kupper, L.L., 1995. The relationship between environmental monitoring and biological markers in exposure assessment. Environ. Health Perspect. 103, 49–53.

Remer, T., Fonteyn, N., Alexy, U., Berkemeyer, S., 2006. Longitudinal examination of 24-h urinary iodine excretion in schoolchildren as a sensitive, hydration status-independent research tool for studying iodine status. Am. J. Clin. Nutr. 83, 639–646.

Remer, T., Neubert, A., Maser-Gluth, C., 2002. Anthropometry-based reference values for 24-h urinary creatinine excretion during growth and their use in endocrine and nutritional research. Am. J. Clin. Nutr. 75, 561–569.

Saghir, S.A., Mendrala, A.L., Bartels, M.J., Day, S.J., Hansen, S.C., Sushynski, J.M., Bus, J.S., 2006. Strategies to assess systemic exposure of chemicals in subchronic/chronic diet and drinking water studies. Toxicol. Appl. Pharmacol. 211, 245–260.

Scher, D.P., Alexander, B.H., Adgate, J.L., Eberly, L.E., Mandel, J.S., Acquavella, J.F., Bartels, M.J., Brzak, K.A., 2007. Agreement of pesticide biomarkers between morning void and 24-h urine samples from farmers and their children. J. Exp. Sci. Environ. Epidemiol. 17, 350–357.

USEPA, 1999. Toxicological Profile for Ethylene Glycol Monobuty Ether (EGBE) (CAS No. 111-76-2). In Support of Summary Information on the Integrated Risk Information System (IRIS).

USEPA, 2002. A Review of the Reference Dose and Reference Concentration Processes. Risk Assessment Forum. EPA /630/P-02/002F.

Yager, J.W., 1990. Development of biological monitoring as a tool for exposure assessment. In: Rappaport, S.M., Smith, T.J. (Eds.), Exposure Assessment for Epidemiology and Hazard Control. Lewis Publishers, Inc., Chelsea, MI, pp. 115–130 (Chapter 8).