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Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation

NOTICE

Office of the Science Advisor
Risk Assessment Forum
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ABSTRACT

This document describes the U.S. EPA's approach for developing quantitative factors for extrapolating effect levels from animals to humans and to address human variability. These extrapolations have been covered by the uncertainty factors UF_A and UF_H, respectively. In the absence of quantitatively-valuable data, default values for these uncertainty factor values may be applied. However, informative data that describe variability in chemical distribution (toxicokinetics, TK) and dose-response (toxicodynamics, TD) should be first considered. This document describes the separation of UF_A and UF_H into TK and TD components and describes the process for identifying pertinent data useful for quantifying inter- and intraspecies differences to serve as the basis for nondefault, data-derived extrapolation factors (DDEFs). Key considerations include identifying a tissue concentration associated with a given response level, and identifying and measuring a biological response associated with the corresponding toxicity. Interspecies TK variability is quantified on the basis of doses or concentrations that produce the same tissue concentration in animals and humans; intraspecies TK variability is defined as differences in tissue concentration attained from the same human exposure; TD variability is defined on the basis of doses or concentrations that produce the same response. This approach is consistent with the approach for deriving reference concentration (RfC) values; it represents a point in the continuum of approaches that includes default approaches, categorical default values (e.g., body-weight scaling), and integrated biologically based dose-response models.

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LIST OF ABBREVIATIONS

ADME	absorption, distribution, metabolism, elimination; toxicokinetics
AUC	area under the curve
AUC_{sens}	area under the curve at a percentile of interest in the sensitive human population
AUC_A	area under the curve in the animal
AUC_{gen}	area under the curve at a measure of central tendency in the general human population
AWQCD	Ambient Water Quality Criterion document
BBDR	biologically based dose response
BMD	benchmark dose
BMD_{10}	benchmark dose corresponding to a 10% increase in response
BMDL	lower 95% confidence limit of the benchmark dose
$BMDL_{05}$	benchmark dose corresponding to a 5% increase in response
Cl	clearance
Cl_{int}	intrinsic clearance
C_{max}	maximum concentration
CSAFs	chemical-specific adjustment factors
CYP	cytochrome P450
DDEFs	data-derived extrapolation factors
ED_{10}	effective dose producing a 10% response rate
EGBE	ethylene glycol monobutyl ether
HED	human equivalent dose
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
K_m	Michaelis constant, substrate concentration at rate of 1/2 V_{max}
LOAEL	lowest-observed-adverse-effect level
MOA	mode of action
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
PBPK	physiologically-based pharmacokinetic
POD	point of departure
RfC	reference concentration
RfD	reference dose
TD	toxicodynamics
TK	toxicokinetics
U.S. EPA	U.S. Environmental Protection Agency
UF	uncertainty factor
UF_A	interspecies uncertainty factor
EF_{AD}	toxicodynamic component of interspecies extrapolation factor
EF_{AK}	toxicokinetic component of interspecies extrapolation factor
UF_H	intraspecies uncertainty factor

LIST OF ABBREVIATIONS (continued)

EF_{HD}	toxicodynamic component of intraspecies extrapolation factor
EF_{HK}	toxicokinetic component of intraspecies extrapolation factor
V_{max}	theoretical maximal initial velocity
WHO	World Health Organization

PREFACE

This guidance document is intended to help U.S. Environmental Protection Agency (EPA) risk assessors take a data-based approach to nonlinear low-dose extrapolation. It describes data recommendations and methods or procedures to calculate data-derived extrapolation factors (DDEFs) for chemicals for inter- and intraspecies extrapolation. While serving the same purpose as uncertainty factors, these extrapolation factors are based on data directly pertinient to the chemical of interst, rather than having their basis on default assumptions about inter- and intra-species variability. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (or "*Cancer Guidelines*") (U.S. EPA, 2005) describes the EPA's current practice of examining all relevant and available data first when performing a risk assessment. When chemical—and/or—site-specific data are unavailable or insufficient, the EPA uses default assumptions or processes in order to continue with the risk assessment. Under this practice, EPA guidance suggests invoking defaults only after the data are determined not to be usable at a particular point in the assessment. Both the *Cancer Guidelines* and the EPA document, *An Examination of EPA Risk Assessment Principles and Practices: a Staff Paper* (U.S. EPA, 2004), note that this is a shift in paradigm from the older practice of assuming that default values or processes will be used unless there are sufficient data to warrant a departure from the default. Neither document describes specific approaches to using data rather than default uncertainty factors in low-dose extrapolation. This document, *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation,* describes those approaches and processes.

The members of the Risk Assessment Forum Technical Panel on Data-Derived Extrapolation Factors emphasize that the information offered here is neither a checklist nor a set of requirements that must be met in order to perform inter- and intraspecies dose extrapolation. It presents guidance and codifies many of the best practices available when evaluating data for the purpose of inter- and intraspecies extrapolation factor development. This will contribute to consistency in the EPA processes and decisions.

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1. INTRODUCTION

1.1. BACKGROUND

Risk assessment is the U.S. Environmental Protection Agency (EPA or Agency)'s key process for deriving scientific information for making sound decisions on managing risks to human health and the environment (U.S. EPA, 2004). No risk assessment can reflect true risk with absolute certainty, so it is important that uncertainties be handled in a predictable, scientifically defensible way that is both consistent with the EPA's mission and responsive to the needs of decision makers (U.S. EPA, 2004). This involves decreasing uncertainty in estimates wherever possible, defining uncertainty and variability in estimates, and quantifying the uncertainty when feasible. Historically, the Agency has used default uncertainty factors to compensate for a lack of information. As science has advanced, however, there has been a growing effort to increase reliance on the available data to modify the values for these uncertainty factors (IPCS, 2005); this guidance describes an approach for using such information for developing Data-Derived Extrapolation Factors (DDEFs).

An Examination of EPA Risk Assessment Principles and Practices: a Staff Paper (U.S. EPA, 2004) notes that the EPA has published a number of documents that provide direction in describing uncertainty in risk estimates including the following:

- *Risk Characterization Handbook* (U.S. EPA, 2000)
- *Guiding Principles for Monte Carlo Analysis* (U.S. EPA, 1997)
- May 1997 *Policy for Probabilistic Analysis* (U.S. EPA, 1997a, b)
- *Risk Assessment Guidance for Superfund: Volume III—Part A, Process for Conducting Probabilistic Risk Assessment* (U.S. EPA, 2001a)

Given that risk assessors never have a complete data set, it is accepted practice to use default values and processes in order to allow a risk assessment to proceed in the absence of data. The EPA uses the definition of default assumption articulated by the National Research Council (NRC): "the option chosen on the basis of risk assessment policy that appears to be the best choice in the absence of data to the contrary" (NRC, 1983). The NRC, in its report *Science and*

Judgment in Risk Assessment (NRC, 1994), supported EPA's use of defaults as a reasonable way to deal with uncertainty. That report stated that the EPA should have principles for choosing default options and for judging when and how to depart from them.

Since then, the EPA now initiates the process of choosing a method for developing uncertainty factors by evaluating the available data—guidance suggests invoking default values only when data are unavailable or insufficient. This contrasts with the previous position of using the strength of the data as the basis for moving away from default values for uncertainty factors. Specifically, the 2005 *Guidelines for Carcinogen Risk Assessment* (or "*Cancer Guidelines*") (U.S. EPA, 2005) state *"these cancer guidelines view a critical analysis of all of the available information…as the starting point from which a default option may be invoked if needed to address uncertainty or the absence of critical information."* Thus, while risk assessors have generally tried to make maximum use of available data, the shift away from standard default assumptions was codified as EPA science policy with the publication of the 2005 *Cancer Guidelines.* Evaluating the available data will improve the scientific basis of risk assessments when data are sufficient for refining uncertainty factors (UFs). In cases where data are not sufficient, hazard and risk characterizations will be improved, and data needs can be noted and potentially filled in the future (Murray and Andersen, 2001; Meek, 2001; Meek et al., 2001; Bogdanffy et al., 2001).

1.2. PURPOSE AND SCOPE

U.S. and international efforts have improved the scientific basis for human health risk assessments by increasing the use of mechanistic and kinetic data. For example, the EPA's 2005 *Cancer Guidelines* (U.S. EPA, 2005) emphasize the use of mode-of-action (MOA) information in characterizing potential health effects of exposure to environmental agents. International efforts, including those by the International Life Science Institute (ILSI) and the World Health Organization (WHO)'s International Programme on Chemical Safety (IPCS), have developed frameworks for evaluating animal data to determine the human relevance of described MOAs (Boobis et al., 2008; Seed et al., 2005; Sonnich-Mullin et al., 2001). These documents guide the qualitative and quantitative evaluation of the relevance of a particular animal model of action in humans and discuss the use of in vivo and in vitro data when considering animal-to-human

extrapolation. The 2005 *Cancer Guidelines* (U.S. EPA, 2005), and other documents like IPCS's chemical-specific adjustment factors (CSAFs) guidance (IPCS, 2005), the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), and *An Examination of EPA Risk Assessment Principles and Practices:Staff Paper Prepared for the U.S. EPA by Members of the Risk Assessment Task Force* (U.S. EPA, 2004) also encourage the use of sophisticated models like physiologically based pharmacokinetic (PBPK) and biologically based dose-response (BBDR) models in interspecies extrapolation.

This document deals specifically with the development and use of data-derived factors in the calculation of nonlinear low-dose estimates, or safety assessments. The goal of DDEFs is to maximize the use of available data and improve the scientific support for a risk assessment. The processes described herein have benefited from the continuing discussion in the scientific community on ways to replace the 10-fold uncertainty factors (10× UFs) that have historically been used in deriving safety assessments such as reference doses (RfDs), minimal risk levels, and acceptable daily intakes. WHO's IPCS guidance for deriving CSAFs was finalized in 2005. This CSAF guidance describes approaches for use of kinetic and mechanistic data to refine inter- and intraspecies extrapolation factors. The IPCS guidance is largely based on analyses by Renwick (1993) and Renwick and Lazarus (1998), which describe the use of toxicokinetic and toxicodynamic data as a means of replacing the traditional 10× UFs for human sensitivity and experimental animal-to-human extrapolation. This data-derived approach assigns values for toxicokinetic and toxicodynamic differences as replacements for each traditional 10× UF. Important distinctions between IPCS (2005) and the present EPA guidance are that IPCS restricts toxicokinetic evaluations to the central compartment, disallowing local tissue metabolism to be quantified as part of the toxicokinetic processes; division of the animal to human extrapolation unevenly, attributing a greater fraction of default uncertainty to TK than to TD; and a general level of depth.

The current document describes the EPA's approach to calculating extrapolation values based on data; these are called *data-derived extrapolation factors* (*DDEFs*). DDEFs are similar in concept to IPCS/WHO's CSAFs in that the standard extrapolation factors are separated into toxicokinetic (TK) and toxicodynamic (TD) components, and kinetic and mechanistic data are used to derive refined interspecies or intraspecies extrapolation factor(s). Conceptually, DDEFs

(and CSAFs) may not be limited to a specific chemical but may also apply to chemicals with common structural characteristics, common MOA, or common toxicokinetic characteristics or determinants. An appendix to this document contains case study examples taken from the Integrated Risk Information System (IRIS) and from program office records. These case studies present the application of principles contained in this document to data and modeling studies for actual chemicals and should serve as instructional aides.

Topics most relevant to the derivation and use of DDEFs are the focus of this document. Thus, there are concepts beyond the scope of this guidance that are not discussed in detail here: approaches for selecting critical effects; establishing key events in an MOA analysis;[1] deriving points of departure; performing benchmark dose analysis; and developing and evaluating PBPK and BBDR models. In addition, this document deals only with DDEF for the areas of inter- and intraspecies extrapolation; there is no discussion of factors that have been used for other areas of uncertainty or variability (e.g., duration, database deficiencies, or lack of a no-observed-adverse-effect-level [NOAEL]).[2]

[1] Mode of action (MOA) refers to a series of key, determinant, and necessary interactions between the toxicant and its molecular target(s) that lead to the toxic response. Refer to Section 2.2.4.1 for further information.

[2] Note:The Food Quality Protection Act (FQPA) mandates the use of a presumptive 10-fold factor for the protection of infants and children in addition to inter- and intraspecies factors. This factor can only be modified based upon reliable data. The FQPA factor is not discussed in this document.

2. TECHNICAL CONCEPTS AND PRINCIPLES FOR DDEF

2.1. BACKGROUND

The methodologies of the EPA derivation of reference concentrations (RfCs) and RfDs (the predominant EPA nonlinear approaches) recognize steps for inter- and intraspecies extrapolation, both of which may include the application of uncertainty factors to an experimental result to account for recognized uncertainties in, and variability inherent in, the extrapolations from the experimental data conditions to estimates appropriate to the assumed human scenario (U.S. EPA, 2011, 2002b, 1994, 1993). This document describes an approach to performing inter- and intraspecies extrapolations based on the use of the best available science and data. DDEFs are factors estimated from quantitative data on interspecies differences or human variability (illustrated in Figure 1). DDEFs may consider both toxicokinetic and toxicodynamic properties. These factors can be derived for a single agent or chemical, for a class of chemicals with shared chemical or toxicological properties, and for a group of chemicals that share a mode or mechanism of action or toxicokinetic characteristics. As described below, DDEFs can be calculated using sophisticated toxicodynamic or toxicokinetic models or can be calculated as ratios using key kinetic or dynamic data. With regard to interspecies extrapolation, the EPA currently recognizes a hierarchy of approaches ranging from the preferred approach using PBPK modeling (U.S. EPA, 2006, 1994) down to default approaches for situations for which data do not support an alternate approach, with DDEFs falling intermediate in this hierarchy.

The default approach for the inhalation exposure route involves a combination of application of a categorical dosimetric adjustment factor and a residual uncertainty factor (U.S. EPA, 1994). The dosimetric adjustments are based on the following:

- Anatomic and physiologic differences between species
- Physical differences between particles and gases
- Whether the toxic effect(s) are portal of entry or systemic in nature

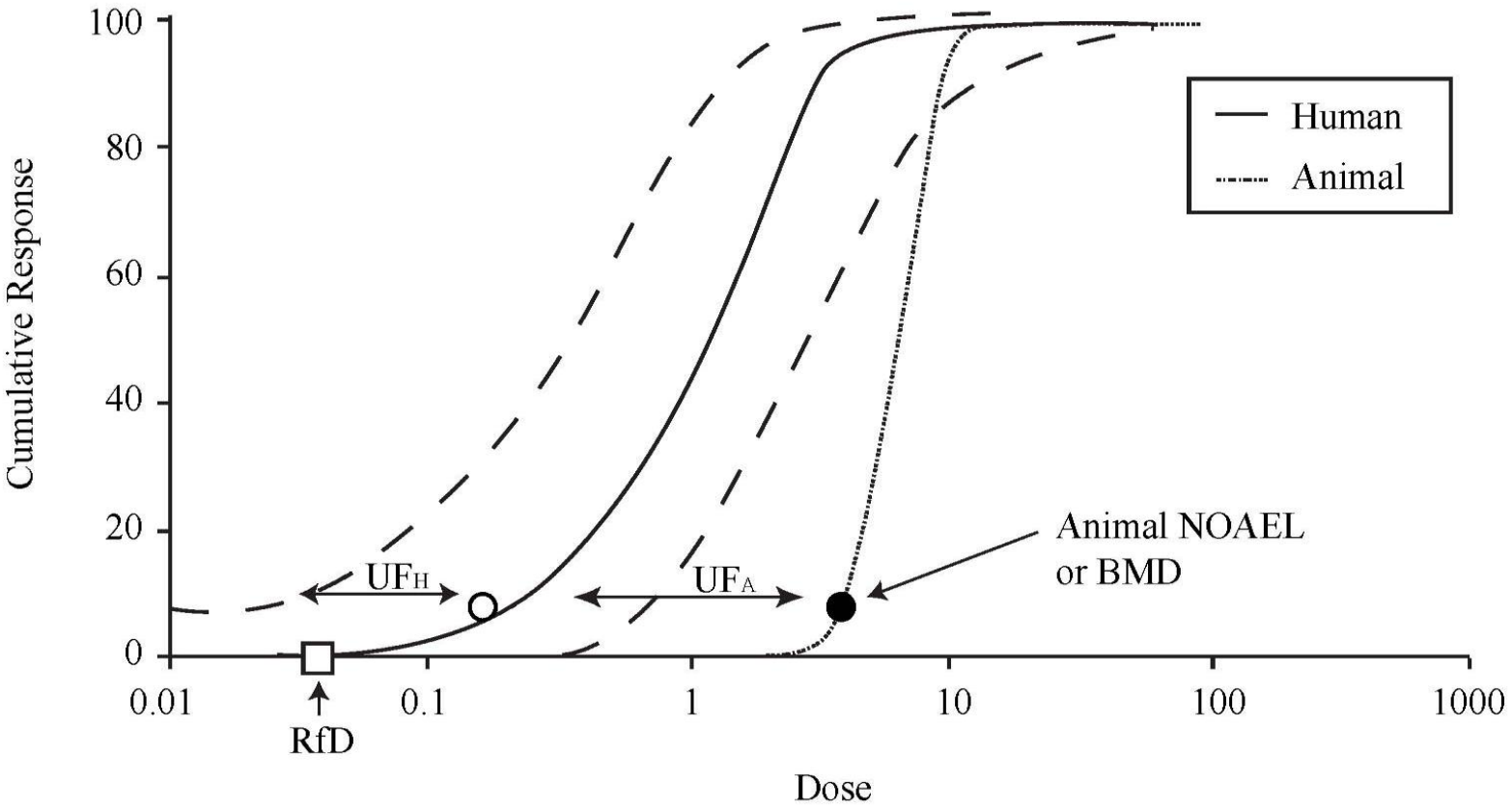

Figure 1. Derivation of RfDs/RfCs using uncertainty factors.

For the oral exposure route, the default approach for interspecies extrapolation involves scaling the applied dosing, according to body weight, to the ¾ power, and a residual uncertainty factor (U.S. EPA, 2011). Apportioning the default values for both inter- and intraspecies extrapolation is based on data for various chemicals. It is generally recognized that toxicokinetic data are more widely available than toxicodynamic data. The magnitude of variation in the available TK data suggests that the interspecies uncertainty factor might be evenly divided between TK and TD components. These values are ½ order of magnitude in value and can be seen in various documents as values of 3, 3.0, 3.16, or 3.2. Regardless of their values, the mathematical combination of two factors of ½ order of magnitude each results in a value of 10 (i.e., $3 \times 3 = 10$). After quantifying TK differences between species, the residual uncertainty factor associated with either route (oral or inhalation) has a default value of 3, which may be modified based on available data (U.S. EPA, 2011, 1994). In accordance with the hierarchy of approaches, when available agent-specific data are supportive of DDEF derivation, a data-derived approach is preferred over using the RfC approach or ¾ body-weight scaling.

2.2. EXTRAPOLATION WITH DDEFs

The foundation of DDEFs is the concept that the toxicity of a particular agent is due to a combination of both toxicokinetic and toxicodynamic factors and that those factors can be quantified in animals and humans. For purposes of this guidance, toxicokinetics (TK) is defined as the determination and quantification of the time course and dose dependency of absorption, distribution, metabolism, and excretion of chemicals (sometimes referred to as pharmacokinetics or ADME). Toxicodynamics (TD) is defined as the determination and quantification of the sequence of events at the cellular and molecular levels leading to a toxic response. There is no clear separation between TK and TD because the processes leading to biological responses include aspects of both—including interactions between TK and TD processes.

2.2.1. Approaches to Deriving DDEFs

The focus of this guidance is on extrapolation from animals to humans, and within the human population. Extrapolation can be accomplished by one of several approaches ranging from the use of highly sophisticated BBDR models to the calculation of relatively simple ratios using TK or TD data describing critical factors in inter- or intraspecies extrapolation. The

following text describes these approaches. In the absence of data for performing sophisticated modeling or for deriving DDEF values, default approaches are used. Figure 2 is a flowchart depicting the decision process used in deriving and applying extrapolation factors.

2.2.1.1. *TK and TD Models*

TK and TD models represent the preferred approach to intra- and/or interspecies extrapolation. They vary in level of complexity from classical compartmental and simple statistical response models to physiologically realistic models of TK and TD processes, up to and including BBDR models. These models provide a quantitative description of the biological processes involved in the toxicokinetics and/or MOA of chemical(s). In these TK and TD models, some measure of the internal dose is related to the external dose and mode of action, respectively.

TK modeling is the process of developing a mathematical description of ADME in a living organism. Two common types of models are (1) data-based classical noncompartmental or compartmental models and (2) PBPK models. Data-based models, also known as classical models, mathematically describe the temporal change in chemical concentration in blood, tissue, or excreta of the species in which the data were generated. The classical models treat the body as a single homogenous or multicompartment system with elimination occurring in a specific compartment; the characteristics of the compartments (number, volume, etc.) are hypothetical in that they are chosen for the purpose of describing the data rather than a priori based on the physiological characteristics of the organism. Due to these characteristics, classical models are used for interpolation, i.e., within the range of doses, dose route, and species in which the data were generated (Renwick, 1994).

PBPK models differ from classical compartmental models in that they are composed of compartments with realistic tissue volumes that are linked by blood flow. Other parameters used in these models account for chemical-specific characteristics that can be independently measured in both humans and laboratory animals (usually using in vitro techniques); these chemical-specific parameters include tissue solubility (i.e., partition coefficients), binding, and metabolism. These models are used to simulate the relationship between applied dose and internal dose. They are more data intensive to develop compared to classical compartmental

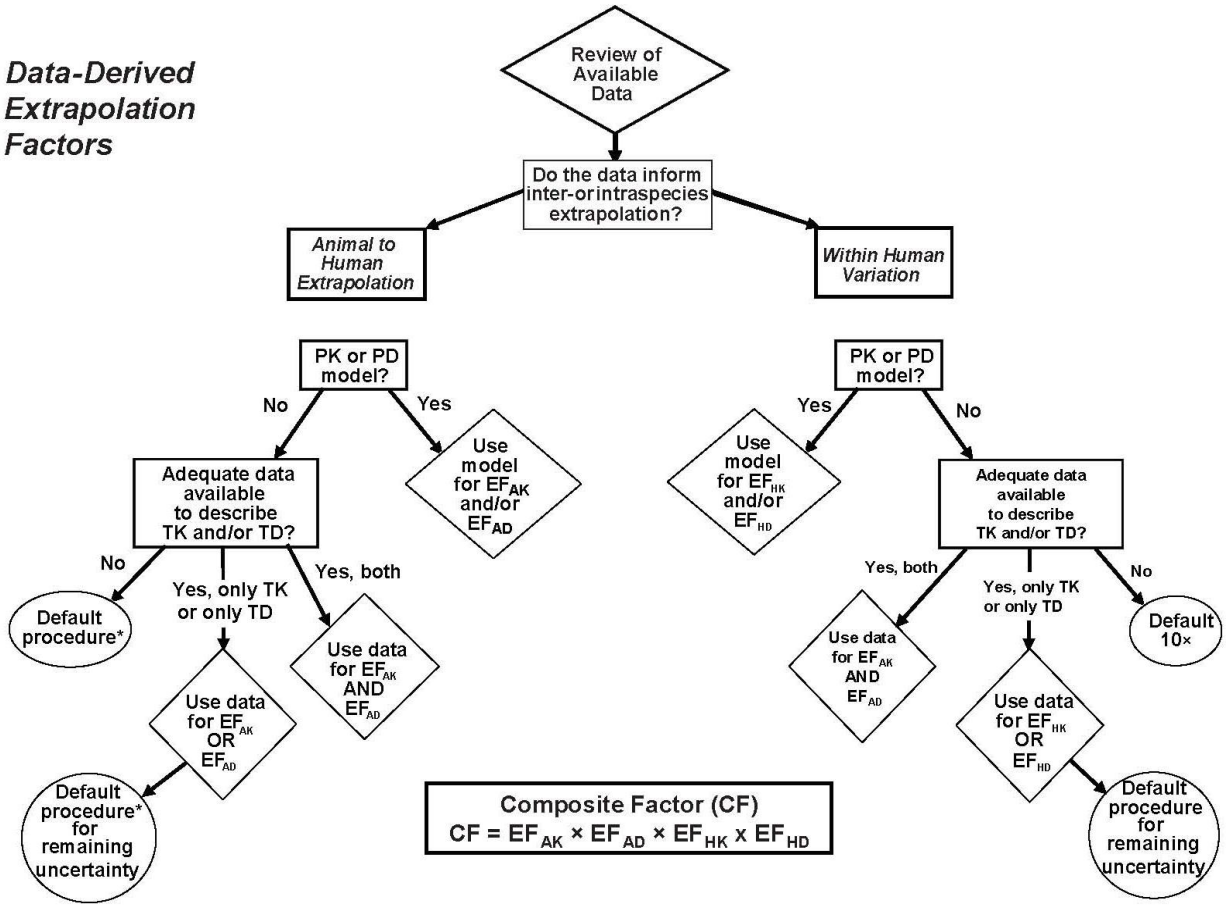

Figure 2. Decision process for DDEFs. The availability of an adequate pharmacokinetic (PK) or pharmacodynamic (PD) model is first considered followed by analysis of the availability of adequate data to describe the toxicokinetics (TK) and/or the toxicodynamics (TD) of the chemical. With the availability of an adequate model or data, data-derived extrapolation factors for intraspecies (EF_{AK}, EF_{AD}) and interspecies extrapolation (EF_{HK}, EF_{HD}) are developed. Such data-derived factors are preferred over default factors. In the absence of an adequate model or data, default factors are used.

*For interspecies extrapolation, the default procedure is ¾ body-weight scaling for oral (U.S. EPA, 2006) and the RfC method (U.S. EPA, 1994) for inhalation to account for potential TK differences with a 3× factor for potential TD differences. The composite factor (CF) accounts for inter- and intraspecies extrapolation and can comprise default or DDEF values for the four extrapolation factor components.

models, but they are advantageous because they can be used for extrapolation (i.e., across dose range, among animal species, between routes of exposure, and across exposure scenarios) (Krishnan and Andersen, 1994; U.S. EPA, 2006).

TD models can be developed when there are sufficient data to both ascertain the MOA and to quantitatively support model parameters that represent rates and other quantities associated with key precursor events in the MOA. A BBDR model describes biological processes at the cellular and molecular levels in such a way as to link target tissue dose with adverse effect; in practice, BBDR models are often described as a combined TK/TD model. These models may be used for extrapolation. However, with adequate understanding of the nature of the response, empirical data describing the dose-response function in relevant species or population groups are sufficient to serve as the basis for DDEF derivation; in these cases, a fully developed TD model may not be required.

DDEF values are extrapolation factors, as opposed to uncertainty factors, per se. DDEF values are quantitatively derived based on TK and/or TD data for the chemical under evaluation. DDEF values are not the same as the default uncertainty factor values, but the values for the DDEF components may sometimes be similar to default values for uncertainty factors. Developing a DDEF value reduces uncertainty and carries with it a change in nomenclature.

2.2.1.2. *Use of Ratios to Calculate DDEF*

In the absence of sufficient data to develop a robust TK or TD model, the risk assessor need not necessarily use default 10× UFs. DDEFs can be calculated as ratios using data from key studies evaluating TK or TD profiles or properties of a particular chemical. Example equations for calculating DDEFs are provided in Table 1 and are described in more detail in Sections 3 (TK) and 4 (TD).

In general, *interspecies* extrapolation involves calculating a ratio of human data for a kinetic or dynamic parameter to animal data for a kinetic or dynamic parameter. Similarly, for *intraspecies* extrapolation, a ratio is calculated using data from the sensitive population and that for the general, or average, population. Data to derive the TK factors may come from in vivo or in vitro studies. For TD, in general, interspecies extrapolation may come from in vivo studies but will often be accomplished with in vitro data in a relevant tissue. When data on toxic effects

Table 1. Example equations used to derive DDEFs

Extrapolation	Toxicokinetics (see Section 3)	Toxicodynamics (see Section 4)
Animal to human (interspecies)	$EF_{AK} = \frac{Dose_A}{Dose_H}$	$EF_{AD} = \frac{Concentration_A}{Concentration_H}$
Within human (intraspecies)	$EF_{HK} = \frac{AUC_{gen}}{AUC_{\%tile}}$	$EF_{HD} = \frac{Concentration_{gen}}{Concentration_{sens}}$

EF_{AK} = extrapolation factor for interspecies extrapolation covering toxicokinetics.
$Dose_A$ = administered or external dose to the animal.
$Dose_H$ = administered or external dose to the human.
EF_{AD} = extrapolation factor for interspecies extrapolation covering toxicodynamics.
$Concentration_A$ = concentration of the agent at the tissue in the animal.
$Concentration_H$ = concentration of the agent at the tissue in the human.
EF_{HK} = extrapolation factor for intraspecies extrapolation covering toxicokinetics.
AUC_{gen} = area under the curve at a measure of central tendency in the general human population.
AUC_{sens} = area under the curve at a percentile of interest in the human population.
EF_{HD} = extrapolation factor for intraspecies extrapolation covering toxicodynamics.
$Concentration_{gen}$ = concentration at a measure of central tendency in the general human population.
$Concentration_{sens}$ = concentration at a percentile of interest in the human population.

are available in humans, these data may be used directly for the point of departure (POD) development, eliminating the need for the interspecies extrapolation. Likewise, they can be used to inform an interspecies factor when the POD is derived from animals.

For DDEFs involving interspecies extrapolation, it is preferred that the ratio be based on data at or near the POD. When sufficient data are available, DDEF values should be calculated for a range of doses near the POD because the shape of the dose-response curve can vary among species. Metabolism and kinetic properties can vary across doses, particularly in the higher dose ranges; thus, using estimates at or near the POD helps avoid introducing significant uncertainty in the DDEF estimate caused by nonlinearity in kinetic properties. Evaluating a range of PODs takes into account the variability of the DDEFs based on the POD selected. The interspecies DDEF should be derived using an estimate of central tendency, such as the mean, median, or mode, depending on the characteristics of the data. It is, however, important to evaluate

variability in the DDEF. Thus, it is recommended that, to the extent possible, the hazard and risk characterizations reflect the upper and lower confidence bounds on the DDEF.

By contrast with interspecies extrapolation, when calculating intraspecies DDEFs, the ratio involves consideration of a measure of central tendency of the general population and lower percentiles of interest (e.g., 1^{st}, 2.5^{th}, or 5^{th}) to represent the sensitive populations. As the needs of risk managers and decision makers vary, it is recommended that a range of percentiles be evaluated and reported in the hazard and risk characterizations.

Toxicokinetic ratios (for either interspecies or intraspecies extrapolation) are based upon the relevant dose metric, such as area under the curve (AUC) and the maximum concentration (C_{max}).[3] Other metrics (e.g., AUC above a threshold) may be used if supported by the data or if relevant for a particular chemical or MOA. For toxicants that bind covalently or cause irreversible damage, especially as a consequence of subchronic or chronic exposure, an integrated measure of dose over time such as AUC is generally used (O'Flaherty, 1989). In the case of effects occurring as a consequence of acute exposure, C_{max} may be more appropriate (Boyes et al., 2005; Barton, 2005). When data on chemical-specific AUC, C_{max}, or clearance (Cl) are not available, a chemical-related physiological parameter (e.g., renal glomerular filtration rate) that is critical to the onset of toxicity or to the MOA may be used.

As Table 1 indicates, there are generally four DDEFs that can be calculated, given sufficient information. Two are for extrapolation from animal data to humans: EF_{AK} is calculated to account for TK variability, while EF_{AD} deals with TD variability. Likewise, there are two factors dealing with variability within the human population: EF_{HK} for TK and EF_{HD} for TD. Table 1 provides example equations for calculating these DDEFs. Section 3 describes specifics for TK factors for interspecies (see Section 3.2) and intraspecies (see Section 3.3) extrapolation. Section 4 describes TD factors for both animal to human (see Section 4.2) and within human (see Section 4.3) extrapolation. Section 5 describes how to combine the EF_{AK}, EF_{AD}, EF_{HK}, and EF_{HD} into the composite UF.

The overall goal of DDEFs is to maximize the use of available data and improve the overall scientific support for a risk assessment. Figure 2 provides a flowchart of the decision process for extrapolation used in deriving DDEFs. As shown in the figure, inter- and

[3] Clearance can be used to calculate this ratio when it can be assumed or demonstrated that the relevant dose metric is AUC or concentration at steady state.

intraspecies extrapolation can be accomplished using a combination of TK or TD models, DDEFs derived from ratios, and/or use of defaults. As described in more detail in Sections 3 and 4, it is important for the hazard and/or risk characterizations to include thorough and transparent discussions of methods and data used to support extrapolation approaches.

2.2.2. Qualitative Considerations

Although in some cases there may not be sufficient data for a quantitative estimate of a DDEF, there may still be information to support a UF different from the default. For example, there may be qualitative evidence that a MOA identified in animals is not relevant to humans. A framework developed by ILSI for evaluating the relevance of an animal MOA can be found in Seed et al. (2005), Meek et al. (2003), and Boobis et al. (2008). The human relevance framework provides a transparent and logical thought process by which animal and human MOA data can be evaluated on both a qualitative and quantitative basis. In these cases, where only qualitative data are available, a thorough weight-of-evidence analysis should be considered with the hazard and/or risk characterization to discuss the derivation of the DDEF along with associated uncertainties in the available database.

2.2.3. Information Quality

Critical evaluation of all data used to support the development of DDEFs is necessary. This includes data used to provide qualitative support for the MOA and choice of dose metric, as well as data used in the quantitative derivation of the DDEF itself. Supporting studies can be evaluated using criteria set forth in various EPA guidance documents, including the recently published 2005 *Cancer Guidelines*, as well as earlier guidelines specific to neurotoxic, reproductive, and developmental endpoints (U.S. EPA, 1998, 1996, 1991). In addition, the general principles outlined in the EPA information quality guidelines are applicable in the critical evaluation of data used to support DDEF development (U.S. EPA, 2002a). The remainder of this section highlights some areas of special emphasis that are particularly relevant to the DDEF derivation process including MOA, uncertainty and variability, and dealing with multiple responding organs or tissues.

Use of secondary data sources is one particular area of concern. Examples of secondary data sources include compilations of pharmacokinetic parameters (e.g., Brown et al., 1997) and

studies cited and summarized in toxicity profiles and review articles. In general, for principal and supporting studies used directly in DDEF derivation, review of the original literature is necessary. In the case of critical assumptions and data, contradictory results from different studies are best resolved by review of the original publications.

Quantitative TK and TD data used in the DDEF-derivation process requires particular attention to the appropriateness of the study design, the analytical methodology used, and the statistical analysis of the data. Consideration of appropriate study design extends beyond simply verifying that the methods used were adequate for the goals of the study; it also encompasses consideration of the relevancy of the animal species or in vitro test system to evaluate MOA. Relevance can be assessed in both qualitative and quantitative terms. For example, if there is a lack of species concordance (i.e., a particular TK or TD process does not occur in humans) or effects occur only under physiologically unrealistic conditions or not in the tissue evaluated, then its relevancy is questionable and uncertain. Criteria used in arriving at such a determination have been published for both the more general case (Seed et al., 2005) and particular endpoints including various forms of rodent cancer (Proctor et al., 2007; Maronpot et al., 2004). Particular considerations relevant to the use of in vitro data are discussed below. Another important factor in terms of relevancy is consideration of whether the TK or TD response represents a uniquely susceptible tissue, process, or population. This is a critical determinant in evaluating the use of data to describe intraspecies variability.

2.2.4. Additional Considerations

2.2.4.1. *Mode of Action*

Information on MOA can greatly enhance DDEF derivation, even when a complete explication of mechanism is not available. In the 2005 *Cancer Guidelines*, the EPA describes MOA evaluation as the critical information that defines the conditions under which a toxicant causes its effect, the relevance of animal data for hazard identification, and the most appropriate approach to low-dose extrapolation. The 2005 *Cancer Guidelines* also present a framework for evaluating data in support of MOA determination. Major components of this framework include a description of the hypothesized MOA and a discussion of the experimental support for the hypothesized MOA based on modified Hill criteria (U.S. EPA, 2005) for demonstrating associations in human studies.

MOA is defined as a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in toxicity. A key event is an empirically observable precursor step that is itself a necessary element of the MOA or is a biologically based marker for such an element. MOA is contrasted with "mechanism of action," which implies a more detailed understanding and description of events, often at the molecular level, than is meant by MOA (U.S. EPA, 2005).

DDEFs for both TK and TD are endpoint driven—that is, considered in the context of the toxic endpoints most relevant for purposes of the risk assessment. Understanding MOA for the agent(s) of interest helps to ensure that the TK or TD parameter used to derive the DDEF will be robust scientifically. The key events in MOA are likely to identify important metabolite(s) and potential species differences. Moreover, data on key events may be used directly to estimate the EF_{AK} or the EF_{AD}.

2.2.4.2. *Use of In Vitro Data*

In vitro assays play an important role in defining DDEFs; however, care must be taken to avoid taking isolated findings out of context. Consideration of interspecies differences in ADME is essential because the dose to target tissue in any given exposure scenario is a balance among multiple and competing ADME processes. Thus, in vitro data should not be used for quantitative purposes unless interpreted in the context of the intact system. Among the questions to be considered when applying in vitro data to DDEFs are the following:

- Was the toxicologically active form of the agent studied?
- How directly was the measured response linked to the toxic effect?
- Are the biological samples used in the assays derived from equivalent organs, tissues, cell types, age, stage of development, and sex of the animals/humans in which the target organ toxicity was identified?
- What is the range of variability (e.g., diverse human populations and lifestages) that the biological materials cover?[4]

[4] Quality (purity, viability, source) of the samples is of particular concern, with biological materials derived from human organ donors.

- If the effect occurs or can be measured in several tissues, is the studied tissue or tissue preparation an appropriate surrogate? Or, in situations where the effect is not localized, is the effect consistent across tissues?

- Does the design of the study allow for statistically valid comparisons based on such factors as replication and sample size?

- Was chemical uptake considered when the chemical was applied to the samples so as to give comparable intracellular concentrations across tissues, and similar tissues across species?

- Do the concentrations in the in vitro studies allow for comparison with in vivo conditions?

All of these issues affect the utility of applying in vitro data for risk assessment: a clear discussion of these points helps to clarify the appropriateness of the information used for deriving DDEFs.

Textbox 1

Variability refers to true heterogeneity or diversity. This may be due to differences in exposure as well as differences in response. Those inherent differences are referred to as variability. Differences among individuals in a population are referred to as interindividual variability, while differences for one individual over time are referred to as intraindividual variability.

Uncertainty occurs because of lack of knowledge. It is not the same as variability. Uncertainty can often be reduced by collecting more and better data, while variability is an inherent property of the population being evaluated. Variability can be better characterized with more data but cannot be eliminated. Efforts to clearly distinguish between variability and uncertainty are important for both risk assessment and risk characterization.

Source: U.S. EPA (2002b).

2.2.4.3. *Uncertainty and Variability*

The application of the inter- and intraspecies UFs attempts to account for both the variability (true heterogeneity) and uncertainty (lack of knowledge) the in the data available (see Textbox 1, U.S. EPA, 2002b). The DDEFs described in this document evaluate variability within the data. Evaluation of the sources and magnitude of uncertainty is appropriate (U.S. EPA, 2005, 2001, 1997a, b). Quantitative uncertainty analyses may be undertaken but are not presented in this document. When quantitative approaches are not feasible, qualitative uncertainty analyses may be developed. As is consistent with the 2005 *Cancer Guidelines*: "*a default option may be invoked if needed to address uncertainty or the absence of critical information."*

2.2.4.4. *Multiple Critical Effects*

For some toxicants, multiple critical effects may be identified during hazard identification. In some cases, these effects may be the result of a single MOA. However, for others, the critical effects may have different or unknown MOAs. It is possible that the uncertainty and/or variability associated with the TK and/or TD of each effect may differ, resulting in different DDEFs. The results generated for the multiple responding tissues/organs, particularly if multiple MOAs are operational or MOA is unknown, should be presented for comparison (for example, in a table that is accompanied by a discussion of the methods used). Unless there is scientific support for doing so, it is important not to mix DDEFs derived for one tissue or one MOA with DDEFs derived from a different tissue. For example, DDEF values for kidney effects may not apply to liver effects.

2.2.4.5. *Screening-Level vs. Refined Risk Assessments*

Extrapolation is most scientifically robust when data are first evaluated prior to the use of defaults. However, with a multitude of types of data, analyses, and risk assessments, as well as the diversity of needs of decision makers, it is neither possible nor desirable to specify step-by-step criteria for decisions to invoke a default option. Some risk assessments may be limited by time or resource constraints. Other risk assessments may provide only screening level evaluations. In these cases, the risk assessment may be more likely to resort to one or more default assumptions. On the other hand, risk assessments used to support significant risk management decisions will often benefit from a more comprehensive assessment.

3. DDEFs BASED ON TOXICOKINETICS (TK)

3.1. GENERAL CONSIDERATIONS

Toxicokinetics is concerned with delivery of the biologically active chemical species to the target tissue of interest. Data on tissue concentrations of toxicants or clearance rates of toxicant removal serve as the basis for deriving extrapolation factors foor toxicokinetic components. This section provides a discussion of factors common to derivation of both inter- and intraspecies uncertainty factors to account for TK variability. Data on the quantitative differences in the TK between animals and humans are used for interspecies extrapolation (EF_{AK}); differences in susceptibility within the human population are used for the intraspecies extrapolation (EF_{HK}). Thus, the factor EF_{AK} accounts for extrapolation from laboratory animals to the general human population. The EF_{HK} factor accounts for the variation in the dose/exposure-response relationship between the general human and potentially susceptible human individuals or groups. Note, the term *susceptible* is also used to describe *sensitive* individuals or groups, as these two terms are often used interchangeably, and no convention for their use is widely accepted (U.S. EPA, 2004). Developing a DDEF for TK requires knowledge about the relationship between external dose and internal (target tissue) concentrations. This information can come from studies in which tissue concentrations are observed, both types of data are recorded, or can come from adequate TK models, which expand the range of confidence from that of the empirical observations. TK models, especially PBPK models, represent an important tool through which in vitro observations can be interpreted in the context of the intact system. As such, they represent an advantageous means to evaluate the impact of studies (especially those using human tissues) conducted in vitro.

The TK portion of each factor (EF_{AK}, EF_{HK}) is combined with the corresponding TD factors to assemble the composite extrapolation factor (see Section 5). Where the data are not sufficient to derive a DDEF for TK, other approaches can be considered for EF_{AK} or EF_{HK}. For example, the RfC approach (U.S. EPA, 1994) when evaluating inhalation data or ¾ body-weight scaling, or a default as described in Figure 2.

Important questions to address for TK are given below:

- What is/are the critical effect(s) and POD being used for this assessment?

- What is the MOA or mechanism for that toxicity? Have the key events been identified and quantified? Do these key events identify important metabolic steps?

- Are the process of absorption, distribution, metabolism, and elimination of the chemical well characterized? Do animals and humans metabolize the chemical(s) in a similar way (qualitatively and quantitatively)?

- Are there data in human populations describing variation in important kinetic parameter(s) for this chemical(s)? Do these data identify a susceptible population(s) or lifestage(s)? Can the degree of this susceptibility be estimated?

TK data may be developed empirically or through compartmental or physiologically based TK models. Section 2.2.2 describes how data, models, and approaches are evaluated for their appropriateness. For each critical effect identified for a particular agent, separate DDEF analyses are conducted for EF_{AK} and EF_{HK}. As such, data for multiple susceptible tissues/endpoints can be evaluated, concentrating on those tissues that demonstrate adverse responses near the POD for the critical effect.

3.1.1. Dose Metric

Dose metric is a term used to identify a measure of the internal dose that is associated with the health outcome of interest. It describes target tissue exposure in terms of the toxic chemical moiety (parent or metabolite) and is expressed in appropriate time-normalized terms. For example, acute effects are often most related to peak concentrations, whereas effects occurring following a prolonged exposure are often best correlated with time-normalized (e.g., area under the concentration-time curve) measures of exposure. The choice of the dose metric is an important component in TK extrapolations. This choice depends on whether toxicity is best ascribed to a momentary or transient tissue exposure or a cumulative dose to target tissue. For a given chemical, the appropriate dose metric will also be determined by, and can vary with, both the duration of exposure and the adverse effect of concern (U.S. EPA, 2006). Selection of an appropriate dose metric based upon specific endpoints involves several elements including those described in more detail below:

- Duration of exposure and effect;

- Identification of the active chemical moiety;

- Selection of the organ or tissue group in which some measure of internal dose is desired; and

- Selection of the measure of exposure that best correlates with toxicity.

Whether an adverse effect is a consequence of acute- or chronic-duration exposure impacts the choice of dose metric. For acute, reversible effects (e.g., sensory irritation, narcosis), a measure of instantaneous or peak tissue exposure such as C_{max} may be the most appropriate dose metric (Alarie, 1973; Boyes et al., 2005). For chronic effects, in the absence of MOA information to the contrary, it is generally assumed that some integrated cumulative measure of tissue exposure to active toxicant is the most appropriate dose metric, e.g., area under the curve (AUC). Alternative choices such as amount of chemical or rate of metabolite production can be used as appropriate for a particular agent or MOA (U.S. EPA, 2006). For example, there may be a case where a temporally large influx of active chemical to a target site in a relatively short period of time (peak exposure) is observed, in which case, a less commonly used metric such as time above a critical concentration (TACC) may be most appropriate. In such an instance, the data and rationale in support of a particular dose metric need to be presented.

Clearance, while not typically considered a dose metric, can be useful in DDEF derivation. Clearance is mathematically inversely related to AUC (e.g., AUC = dose/clearance); thus, differences in clearance values can be used in calculation of ratios. When metabolism represents the primary or sole clearance mechanism, either of two clearance models may be applicable. Intrinsic clearance (Cl_{int}) has been used for interspecies scaling of administered doses in drug development (Houston and Carlile, 1997). Cl_{int} is calculated as V_{max}/K_m, and is in units of volume cleared of the substrate per unit time. V_{max} is the theoretical maximal initial velocity of the reaction, and K_m is the substrate concentration driving the reaction rate at one-half V_{max}. Cl_{int} can be extrapolated to the whole body with knowledge of protein binding and the recovery of the protein or cellular or subcellular fraction used in the in vitro investigations (Carlile et al., 1997). Hepatic clearance (Cl_{hep}) is also based on V_{max}/K_m measurements but includes a substrate delivery term, whose value is governed by hepatic blood flow. These measures of clearance differ in that Cl_{int} is not bounded by hepatic blood flow, but Cl_{hep} cannot exceed hepatic blood flow. While metabolic rate constants (V_{max} and K_m) derived from in vitro

data can also be scaled up and incorporated into PBPK models, the use of these clearance models is a simpler approach, useful when an appropriate PBPK model is unavailable. Classical, compartmental TK analyses and measures of clearance are best suited for conditions where metabolism represents a detoxication process, when substrate concentration is less than the K_m value, and when metabolism represents the major clearance mechanism.

Whether toxicity is attributable to a parent chemical, a metabolite, or some combination of metabolites is a critical consideration. The active chemical moiety can be identified through studies in which the toxicities induced by the parent chemical and metabolite(s) are compared or from the results of studies using enzyme inhibitors and/or inducers. In vitro studies can also be quite useful in this regard under appropriate conditions (see Sections 2.2.3.2 and 3.1.3). Quantifying differences in dosimetry can be difficult when metabolic pathways become complex (e.g., where competition among pathways may be concentration dependent). If the metabolic pathway bifurcates and the identity of the bioactive metabolite(s) are unknown or unquantifiable, determination of the appropriate dose metric can be highly uncertain.

The organ or tissue group where the toxic effects occur is ideally the site from which estimates of internal dose (tissue concentration) are generated. In practice, this information may be unavailable in the absence of an appropriate PBPK model. It may be necessary to use absorbed dose of the parent chemical as a surrogate measure of internal dose. Another surrogate dose metric is measurement of parent chemical or active metabolite in circulating blood if the relationship between target tissue dose and blood is known or can be reliably inferred from experimental data. Some data have demonstrated that blood:air partition-coefficient values may vary appreciably between species but that tissue:air (e.g., liver:air) partition coefficients are similar among mammalian species (Thomas, 1975). It seems reasonable to use the cross-species similarity in the primary determinant of diffusion from blood into tissues as a justification to rely on concentrations of the toxicant in blood as a surrogate for tissue concentrations. However, when local tissue bioactivation may be a determinant of the toxic response, this should be given careful consideration. Those issues notwithstanding, measures of internal dose in circulating blood (see IPCS, 2005) may be used as the basis for DDEF derivation under either of these conditions:

- When evaluating interspecies differences, the distribution from blood to sensitive (critical) tissues is shown to be or can be assumed to be the same between animals and humans.

- When evaluating intraspecies differences, the distribution from blood to sensitive (critical) tissues is shown to be or can be assumed to be the same between members of the general human and potentially sensitive human groups.

Because few data are available for concentrations of toxicants in human solid tissues—such as liver, kidney, etc.—compared with data describing toxicant concentrations in human blood, model predictions for solid tissue compartments are less certain than predictions of toxicant concentrations in blood. Partitioning of the active chemical from blood into systemic target tissues may be governed more by physicochemical than by biological processes. This may be considered another basis for relying on data describing the concentration and variability of the biologically active metabolite in the central compartment.[5] For example, the ratio of blood lipid to tissue lipid concentrations may be a key determinant in the diffusion of lipophilic compounds out of blood; however, differences in tissue lipid composition between species may be fairly small compared to differences in blood flow and metabolic activity.

3.1.2. Dose Selection

Because variability in internal dosimetry may be a function of dose, the selection of the external exposure (inhaled concentration or orally ingested dose) is important. In cases where toxicokinetics is nonlinear, the dose selected for the DDEF derivation will impact the magnitude of EF_{AK} or EF_{HK}. Using a dose at or near the POD alleviates some concerns regarding nonlinearities in metabolism. Alternatively, data that show a linear relationship between external dose and internal dose metrics can indicate generalizability of the EF_{AK} or EF_{HK} to doses that may be higher or lower than those used in its calculation.

3.1.3. In Vitro Data

In vitro techniques are important tools in evaluation of toxicokinetics as information can be gathered that are impractical or unethical to collect in the intact animal or humans. However,

[5] The central compartment is defined as blood, plasma, or serum in the systemic circulation. All tissues except those representing the portal of entry are defined as peripheral compartments.

it is important when deriving EF_{AK} and EF_{HK} to consider interspecies differences in ADME. In vitro data should used for quantitative purposes only when interpreted in the context of the intact system, as discussed in Section 2.2.4.2. Care must be taken to avoid taking isolated findings out of context.

3.2. INTERSPECIES TOXICOKINETIC EXTRAPOLATION (EF_{AK})

This section provides a discussion of the quantitative differences in the TK between animals and humans that are used for interspecies extrapolation (EF_{AK}). In this process, toxicokinetic differences between species are characterized as the ratio of applied doses in the test species of interest and humans that result in the same level of the internal dose metric (see Figure 3). These values for the dose metric may be calculated from the external doses actually used in the dose-response evaluations, or by normalizing the dose metric to account for administered dose (e.g., correcting AUC for each species by dividing the AUC by the external dose) when the relationship between the values for the dose metric value and the applied dose are linear in the range of extrapolation. Illustrative case studies are included in the appendix.

3.2.1. Considerations for EF_{AK}

Extrapolation of TK data obtained in animal models to humans (EF_{AK}) is evaluated for each potential critical effect of interest. Data for multiple susceptible tissues/endpoints are evaluated, concentrating on those tissues that demonstrate adverse responses near the POD for the critical effect. This analysis includes consideration of MOA, identification of the active chemical agent for this particular effect, and determination of the appropriate dose metric. This is evaluated as a weight-of-evidence approach emphasizing both qualitative and quantitative evidence. An important part of this process is evaluating concordance of metabolic processes between the animal model and humans. An additional consideration is whether the kinetic data are from a "typical" or average adult animal as opposed to an animal model system that may be unusually sensitive for a particular effect (e.g., metabolic knockout).

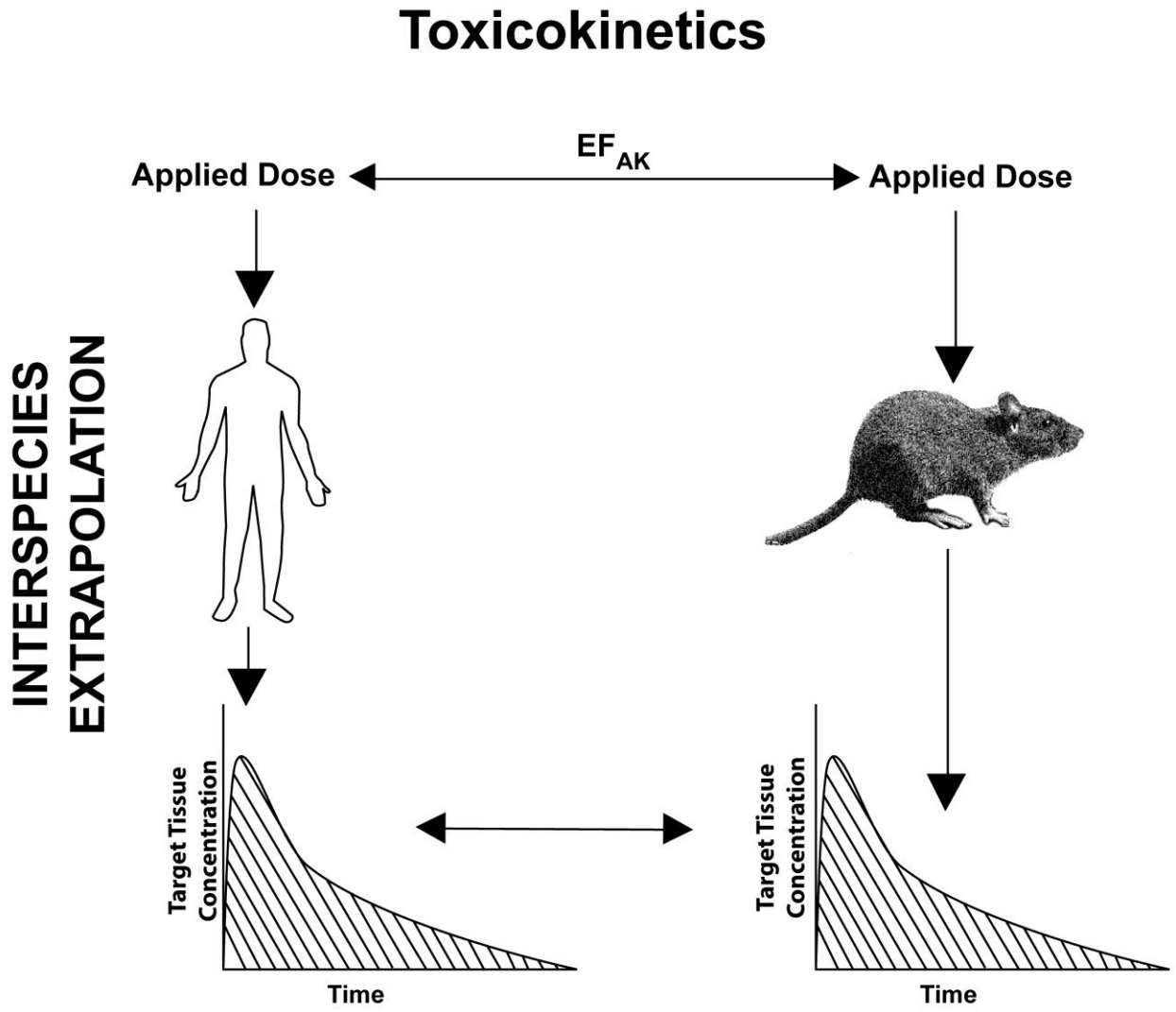

Figure 3. Interspecies toxicokinetics. In keeping with guidance set for inhaled substances (U.S. EPA, 1994), interspecies differences in toxicokinetics are defined as differences in the external dose producing the same level of the dose metric in the target tissue of interest in test animals and in humans representative of the general population. Because it is the default assumption of the Agency that humans are considered more sensitive than animals, EF_{AK} is the ratio of animal dose:human dose suitable for developing a value consistent with its placement in the denominator (i.e., reference value = POD/UF). For clarity in presentation, EF_{AK} is applied to the external dose in animals.

Furthermore, it is important to assess the relationship of externally applied dose to internal dose metric over the entire range of dose levels used in the critical study. There should be careful attention paid both to measures of central tendency and to variability, particularly in

the range of concentrations or doses close to the point of inflection (where the shape or slope of the dose response curve changes) because of potential nonlinearities in metabolism.

3.2.2. Computation

For TK extrapolation, the goal is to determine differences in dosimetry between animals and humans. For interspecies extrapolation, toxicokinetically equivalent exposures (doses) are determined by fixing the internal dose (level of the dose metric at or near the POD) and determining the ratio of external (applied) dose that results in the same level of the dose metric in animals and humans. This approach is consistent with that in the RfC guidance for inhalation toxicants (U.S. EPA, 1994).

3.2.2.1. *Use of TK Models*

A PBPK or other TK model provides the most biologically appropriate approach for evaluating interspecies TK extrapolation. The model is subject to evaluation as previously described (U.S. EPA, 2006). The model can be used in different ways, depending on the model and the circumstances. In some cases, the TK model may be used directly to perform interspecies extrapolation (i.e., to derive a Human Equivalent Concentration or Dose that includes TK considerations), thus alleviating the need for EF_{AK}. In other cases, the TK model may be used to derive EF_{AK}.

3.2.2.2. *Use of Ratios*

When AUC or concentration at steady state is the relevant dose metric, and if advanced TK models are not available, EF_{AK} can be derived using a ratio of doses producing the same AUC value. This is accomplished by identifying external doses associated with the AUC value produced in animals at the point of departure (AUC_A), demonstrated below (see Equation 1 and Figure 4). The human dose that produces the same AUC value as observed in animals (AUC_A) is the TK equivalent dose. In these cases, differences between the animal and the human dose producing the same AUC value in each species are captured as the DDEF. See below:

$$\text{AUC} = \text{Dose/Clearance} \quad (1)$$

$$\text{AUC}_A = D_A / \text{Cl}_A = D_H / \text{Cl}_H$$

Using these data, EF_{AK} is calculated according to Equation 2.

$$EF_{AK} = \frac{D_A \text{ or } \text{Cl}_A}{D_H \text{ or } \text{Cl}_H} \quad (2)$$

Using AUC as an example, the value for the dose metric would be AUC_A, that is the AUC value determined in animals at the POD. Thus, in this example, Equation 2 can be conceptualized as

$$EF_{AK} = D_A \text{ producing } AUC_A / D_H \text{ producing } AUC_A \quad (3)$$

where

AUC_A = area under the curve in the animal

D_A = the animal external dose

D_H = the human external dose.

This is graphically presented in Figure 4.

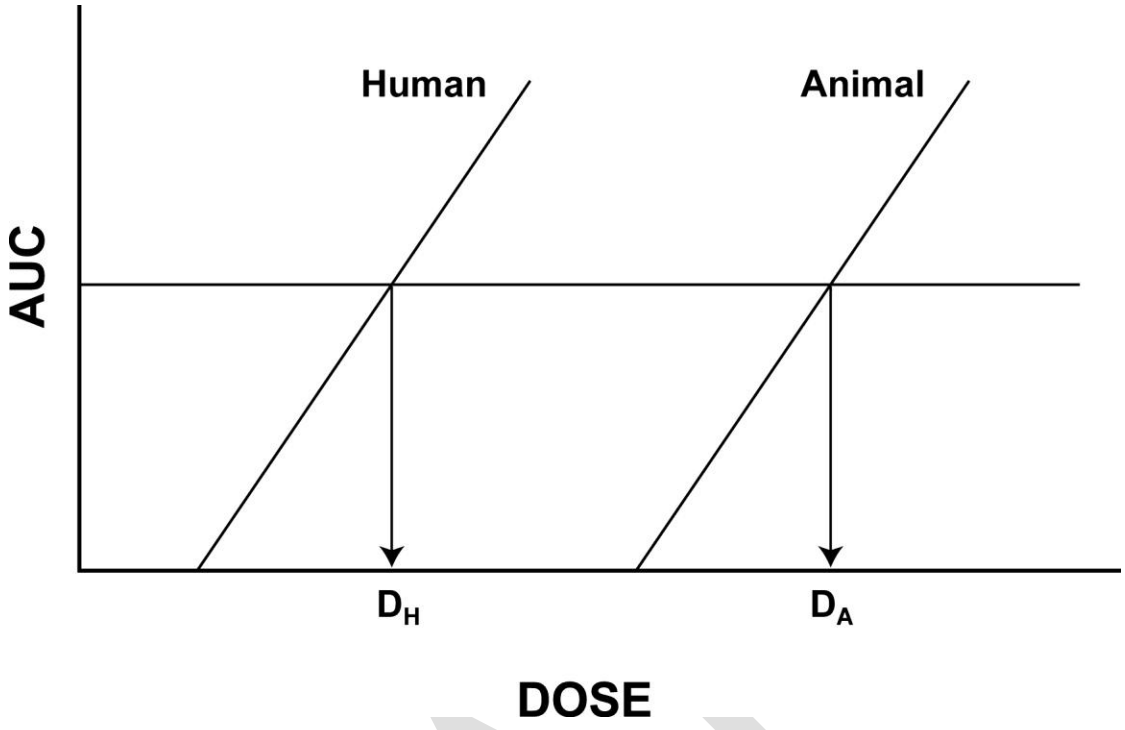

Figure 4. Interspecies extrapolation based on AUC. In this example, AUC is the appropriate dose metric, and the relationship between dose and AUC is determined in animals and in humans. This method can be used to develop a Human Equivalent Dose or Concentration, or in the calculation of EF_{AK}. Calculation of EF_{AK} requires knowledge of applied doses in animals (D_A) and in humans (D_H) that produce the AUC value determined in animals at the point of departure (AUC_A).

Because clearance values are the mathematical reciprocal of internal dose (AUC), they may also be used to calculate a DDEF value. As humans are assumed to be more sensitive than experimental animals (in this instance, they clear toxicants more slowly), the human clearance value remains in the denominator. Calculations using C_{max} are developed in a manner similar to that for AUC.

3.2.3. Relationship to Other EPA Guidance

The development and use of advanced information on tissue dosimetry to serve as the basis for quantitative, nondefault uncertainty factors is consistent with existing EPA guidance and philosophy (U.S. EPA, 2006, 2002). The EPA's Inhalation Reference Concentration Methodology presents a continuum of approaches from complete lack of knowledge to

biologically based dose-response models (U.S. EPA, 1994). There are two approaches that each cover the interval between lack of knowledge and knowledge of target tissue and target tissue concentrations in test species and humans. The first is for reactive (Category 1) gases and inhaled particles that damage portal of entry (respiratory tract) tissues, and the second is for gases that are absorbed and produce their toxicity in tissues bathed by circulating blood (Category 3 gases). For Category 1 gases, toxicity information identifies the affected region of the respiratory tract and species differences in regional respiratory tract surface area and airflow (respiratory rate) serve as the basis to quantify species differences in dosimetry. For Category 3 gases, species differences in the solubility of the compound in blood (the blood:air partition coefficient) serve as the basis upon which to quantify species differences in dosimetry. More recent guidance (U.S. EPA, 2011) on orally encountered toxicants instructs the application of dose scaling by the ratio of species body weights raised to the ¾ power (human BW:animal BW)$^{3/4}$. Each of these guidance documents indicates that their approaches are intermediates, to be superceded when more detailed information on tissue dosimetry can be developed. The subject of the present guidance is the development and interpretation of quantitative toxicokinetic data for the purpose of developing nondefault values for inter- and intraspecies uncertainty/extrapolation.

3.2.4. Conclusions for EF_{AK}

Mathematically, EF_{AK} may be the ratio of the external doses in animals at or near the POD to the value at a central tendency measure of external doses in the general human population that each result in the same level of the dose metric (this represents developing the Human Equivalent Concentration or the Human Equivalent Dose). EF_{AK} values may be calculated for multiple organs/effects. The same PODs should be used for test animals and humans for a given organ/effect. Quantitatively, DDEF values for UF_A components might be less than 1 if humans are less sensitive.

Confidence in EF_{AK} is increased when decisions and calculations are well documented and made transparent. This includes description of toxicity data identifying the target tissue, chemical species, MOA, and species concordance of effects. Data describing the TK, the metabolism of the compound, and the relationship between administered dose and internal dose

are also summarized. Data that show a linear relationship between external dose and internal dose metrics can be specifically reiterated in this description; this will indicate generalizability of the EF_{AK} to doses that may be higher or lower than those used in its calculation. Because animal-to-human differences in target tissue concentrations may not be consistent for all responding tissues or organs, a comparison of POD and DDEF values from multiple affected organs will increase confidence in health-protectiveness of the developed DDEF value for the critical organ. Results can be presented in tabular form for ease of comparison across endpoints.

3.3. INTRASPECIES TOXICOKINETIC EXTRAPOLATION (EF_{HK})

This section provides a discussion of the quantitative differences in the TK among humans for intraspecies extrapolation (EF_{HK}). From a toxicokinetic standpoint, among humans experiencing the same external dose, susceptibility is due to higher target tissue concentrations of the toxicant in some individuals or groups relative to the majority of the human population. TK differences for intraspecies extrapolation are characterized as the ratio of an internal dose metric attained in the general human population to the dose metric from susceptible humans exposed to the same external dose or concentration. Illustrative examples are included in the appendix.

3.3.1. Considerations for EF_{HK}

3.3.1.1. *Susceptible Groups or Individuals*

Potentially susceptible groups/lifestages (e.g., children, elderly, or age-related susceptibility, or those with a disease making them susceptible to a toxicant) can be identified. In some instances, individuals may have a condition or difference in some physiologic or biochemical process that may be a deterministic factor in dosimetry that serves as the basis for assumed sensitivity (e.g., polymorphism of a given metabolizing enzyme). In these cases, the distribution of the dose metric among the population will not have a unimodal distribution (see Figure 5, left panel). In other cases, sensitivity may be distributed throughout the general population, and sensitive individuals may be those in the tail of the distribution (i.e., unimodal; see Figure 5, right panel). Documenting this information and/or these assumptions serves as the basis for selecting a unimodal or a bimodal distribution of sensitivity. The selection of a bimodal or unimodal-based analysis will be a function of the available data and must be made on a

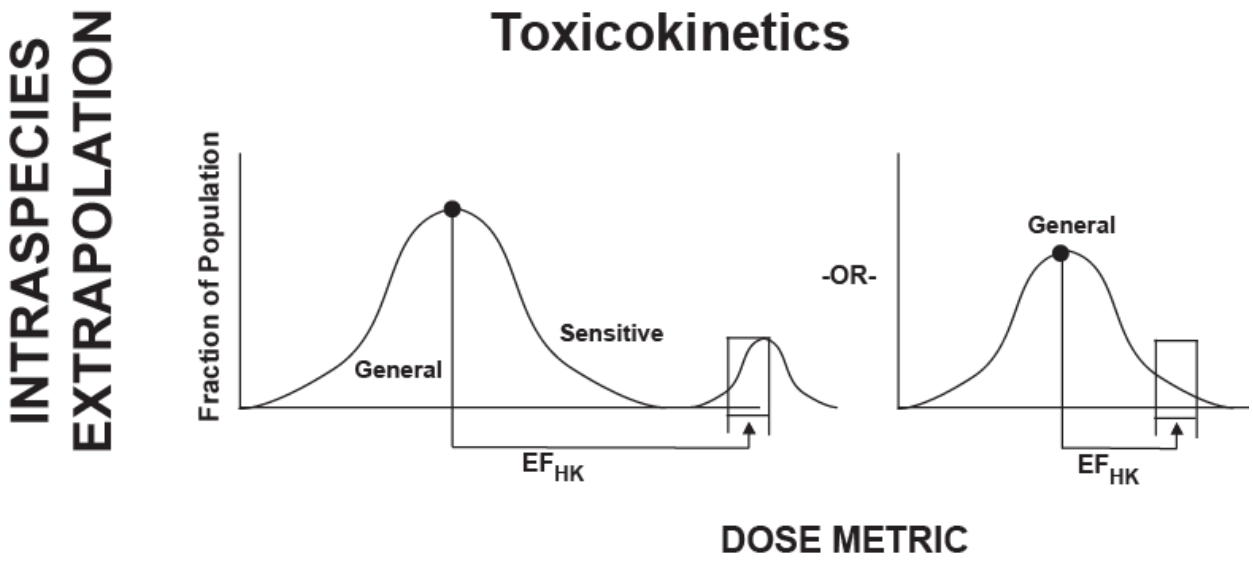

Figure 5. Intraspecies toxicokinetics. From a toxicokinetic standpoint, susceptibility is based on attaining higher target tissue concentrations of the toxicant. For this evaluation, a fixed exposure is studied, and human interindividual variability is measured as differences in the value of the dose metric between sensitive and generally representative population groups. When a group of the population can be identified as potentially susceptible, toxicokinetic data from that group can be compared to the general population (left panel). EF_{HK} should be determined as the ratio of the level of the dose metric at some point in the upper 50% of the distribution (i.e., 95th, 97.5th, 99th percentile) for those deemed sensitive to the level of the dose metric at a central tendency measure of the general population. However, when a separate distribution of values for the susceptible population is not available, then a slightly different analysis is conducted (right panel). In this case, EF_{HK} should be determined as the ratio of the level of the dose metric at a percentile of the population considered sensitive (i.e., 95th, 97.5th, 99th percentile) to the level of the dose metric at a central tendency measure of the general population.

chemical-by-chemical basis. The decision to quantify differences via a unimodal or bimodal analysis should be undertaken on a chemical-by-chemical basis and be based on the strength of the available data for the chemical and for the susceptible population group. One of the challenges in conducting a bimodal analysis will be the availability of data describing chemical disposition and/or physiological and biochemical parameters for the susceptible population group.

3.3.1.2. *Target Tissues*

When responses are observed in several organs at or near the same dose level, a comparison of EF_{HK} values developed for those tissues will be informative. However, the selection of a target organ for calculating human variability other than the one serving as the basis for animal-to-human extrapolation needs to be accompanied with a justification. This also extends to instances in which human studies identify effects in an organ or tissue and variability is assessed in another organ or tissue. The extrapolation approach will be perceived as most consistent when the same tissue or organ is used for each phase of the extrapolation procedure.

3.3.1.3. *Dose Response*

Because variability in internal dosimetry may be a function of dose, the selection of the external exposure (e.g., inhaled concentration or orally ingested dose) is important. Conceptually, the animal POD is first extrapolated to produce a toxicokinetically equivalent human dose or concentration. The DDEF for interspecies differences in TD should be applied to the tissue concentration defining the Human Equivalent Exposure to complete interspecies adjustment. It is this species-adjusted concentration that is most appropriate for application in the evaluation of human interindividual variability.

The basis for comparison of human variability is at the level of the internal, rather than the external dose. Thus, it is important that the relationship between internal and external doses be well characterized. Comparisons of the external doses that produce the same level of the internal dose (between susceptible and nonsusceptible groups) can be substantially complicated when there are nonlinearities in the relationship between external dose and internal concentration. This is particularly difficult when nonlinearity of dose is for one group and not for the other. This complication can be avoided when the comparison is made because differences in internal doses developed from the same external exposure.

Specific differences among humans, particularly those demonstrated in vitro (i.e., intrinsic clearance), are most reliable when they are translated into differences in dosimetry. This may include evaluations of multiple different doses.

3.3.2. Computation

For intraspecies TK extrapolation, differences in dosimetry are characterized for human groups or lifestages by comparison to the general population. Thus, the comparisons are among differences in internal dosimetry (or target site dose) resulting from the same external exposure. To address human variability, some attention must be devoted to identifying a susceptible population group or a fraction of the general population considered to be sensitive. TK data are then analyzed to determine values for the dose metric in the general and sensitive groups.

3.3.2.1. *Use of TK Models*

A PBPK or other TK model provides the most robust approach for evaluating intraspecies TK extrapolation. The model is subject to evaluation as previously described (U.S. EPA, 2006). When an appropriate model is available, it can be used in different ways depending on the model. In some cases, the TK model may directly account for within human variation and/or include data from the sensitive group, thus eliminating the need for EF_{HK}. In other cases, the TK model may be used to derive dose metric values for calculating the value of EF_{HK}.

3.3.2.2. *Use of Ratios*

Where TK models are not available, EF_{HK} can be derived using a ratio (see Equation 4). The value for the dose metric employed for the general population (e.g., AUC_{gen}) would be that level of the dose metric identified from initial studies with animals or humans and further extrapolated to account for interspecies differences in toxicodynamics. The value for the dose metric in sensitive individuals (e.g., AUC_{sens}) should be determined from empirical data or pharmacokinetic modeling.

$$EF_{HK} = \frac{\text{AUC}_{sens} \text{ or } \text{Cl}_{gen}}{\text{AUC}_{gen} \text{ or } \text{Cl}_{sens}} \quad (4)$$

where

EF_{HK} = factor for intraspecies extrapolation covering toxicokinetics

AUC_{gen} = area under the curve at a measure of central tendency in the general human population

AUC_{sens} = area under the curve at a percentile of interest in the sensitive human population

Cl_{gen} = clearance at a measure of central tendency in the general human population

Cl_{sens} = clearance at a percentile of interest in the sensitive human population.

Because clearance values are the mathematical reciprocal of internal dose (AUC), they may also be used to calculate a DDEF value. Because susceptible humans may be assumed to be more sensitive than general humans (e.g., they clear toxicants more slowly), the sensitive human clearance value remains in the denominator.

3.3.3. Conclusions for EF_{HK}

The EF_{HK} is essentially a comparison of internal doses resulting from the same external exposure to both the general population and sensitive individuals. When using an empirical ratio, the EF_{HK} is the ratio of the dose metric value at a percentile of the distribution intended to represent sensitive individuals to the dose metric value at a central tendency measure of the general population. Quantitatively, DDEF values for human interindividual variability cannot be less than one.

The dose selected for quantifying human interindividual variance may have an impact on the magnitude of variability. The dose adjusted from the animal POD is the preferred dose for quantitation of human variability. While DDEF values may be calculated for multiple organs/effects (or even doses), the same external dose needs to be used as the starting point when calculating dose metric values for the sensitive and the general populations.

Documentation should describe the mathematical method to be employed, the type of distribution and percentile(s) of interest; the dose metrics (e.g., AUC of parent compound in kidney), and the target tissues for which EF_{HK} are developed. Confidence in the extrapolation is improved when distribution types are justified or explained.

4. DDEFs BASED ON TOXICODYNAMICS (TD)

4.1. GENERAL CONSIDERATIONS

Toxicodynamics describes the critical interaction of the active chemical moiety with the target site and the ensuing sequence of events leading to toxicity. Data that describe the dose-response relationship serve as the basis for deriving extrapolation factors for toxicodynamic components. This section provides a discussion of factors common to inter- and intraspecies extrapolation for TD. In contrast to TK, which focuses on *differences in internal dosimetry*, TD differences are quantified as *differences in concentration*[6] *producing the same level of response* between animals and humans, as well as among humans. TD evaluations may include multiple response levels, critical effects, key events, or analytical methods. Developing a DDEF for TD requires knowledge about the relationship between an event measured in vitro or in vivo (e.g., receptor binding) and the end result (the adverse health condition). Doses or concentrations producing the measured event can be obtained in vitro or in vivo, but they must be obtained in the tissue of interest or a suitable surrogate. TD models, the most complex of which may be represented by BBDR models, represent an important tool through which predictions may be confidently made to extend the range of empirical observations. Comparisons between animals and humans or to quantify human interindividual differences are made on the basis of doses or concentrations that produce the same level of the same measured response.

Quantitative differences between animals and humans are used for interspecies extrapolation (EF_{AD}), whereas differences in susceptibility within the human population are used for the intraspecies extrapolation (EF_{HD}). Thus, the EF_{AD} is used to extrapolate findings in laboratory animals to the general human population, and the EF_{HD} is used to extrapolate to sensitive human groups and life stages. The TD portion of each (EF_{AD}, EF_{HD}) is combined with the corresponding TK factor to form the composite extrapolation factor.

Although a complete MOA understanding is not required, derivation of a DDEF for TD relies heavily on understanding a MOA for the critical effect(s) identified for risk assessment. Important questions to address include the following:

[6] When using a biologically based dose-response model, differences may be quantified as the ratio of dose metrics in respective species or population groups producing the same level of the response. However, DDEF values for TD may also be quantified as the ratio of in vitro concentrations producing the same level of the response.

- What is/are the critical effect(s) and POD(s) being used for this assessment? (Each of these should be considered in this process.)

- What is the MOA or mechanism(s) for that toxicity? Have the key events been identified? Can they be measured?

- Is the MOA or mechanism(s) in the animal model relevant to humans (qualitatively and/or quantitatively)?

- Are the data on the key events amenable to modeling such that a uniform measure in animals and humans can be derived? If not, do the available data points include a response level that is sufficiently similar in animals and humans?

- Are there data in human populations that describe population variation? Do these data identify a sensitive group(s) or lifestages? Can the degree of this sensitivity be estimated?

4.1.1. Mode of Action

TD extrapolation should be endpoint driven (considered in the context of the toxic endpoints most relevant for purposes of risk assessment). The choice of critical effect should be justified, when possible, based on findings of response in exposed humans. Understanding MOA for the agent(s) of interest helps to ensure that the TD responses used to derive the DDEF will be robust scientifically. These responses could include receptor affinity, enzyme inhibition, and molecular changes, among others. Repair of DNA or tissue damage, biological thresholds, residual function, and other processes that could contribute to nonlinearity in effect are considered and discussed. Experimental systems and measured responses should be the same or comparable.

4.1.2. Relating Response to Dose or Concentration

TD data may be developed from the results of in vivo or in vitro studies. In some cases, in vivo data may be used, but care should be taken to control for the influence of toxicokinetics. If there are existing human data measuring the response, then these data can be used to derive the POD, thereby removing the need for the UF_A. Likewise, if there are data from the most sensitive human group, those data could be used for the risk assessment, and an UF_H may not be needed.

Care should be taken to ensure that kinetic factors do not confound the interpretation of response data. Comparisons based on internal dose metric are preferred over applied dose; that

is, the concentration producing the level of response (rather than applied dose or concentration) is preferred so that TD response may be distinguished from TK differences. Tissue-specific metabolism could also influence the actual target concentrations and must be considered. Blood levels are an acceptable measure of internal dose when it can be shown that they are proportional to concentrations of toxicant in target tissue or biological preparation.

When using in vitro systems, the response measured should be representative of the toxicity; that is, the measured endpoint should be the same as—or highly related to—the critical effect or key event. In cases where the measured response differs, it is important to describe the potential impact of these differences on the final DDEF. When using in vitro data, the comparability of chemical uptake between animal and human tissues should be demonstrated. In vitro results should include data describing the test chemical metabolism by the system used to generate response data.

4.1.3. Range of Doses or Concentrations

The relationship between the doses, tissue concentrations, and/or in vitro concentrations used to derive the DDEF, and those attained in the toxicity studies characterizing the POD, should be characterized. Optimally, the concentrations used in studies characterizing response include the concentration at the POD. This is important because the variability in the response may change with increasing or decreasing dose or concentration. Doses and/or tissue concentrations may also be compared to those expected from environmental exposure; this is part of the consideration of overall relevance of the test system.

4.2. INTERSPECIES TOXICODYNAMIC EXTRAPOLATION (EF_{AD})

This section provides information on calculating TD differences between species. These are characterized as the ratio of the concentrations (concentrations used in vitro or the level of the dose metric) in animals and humans producing the same level of response. Note that the level of comparison is the response, and it is not a comparison of responses produced by the same concentration (see Figure 6). Illustrative examples are included in the appendix.

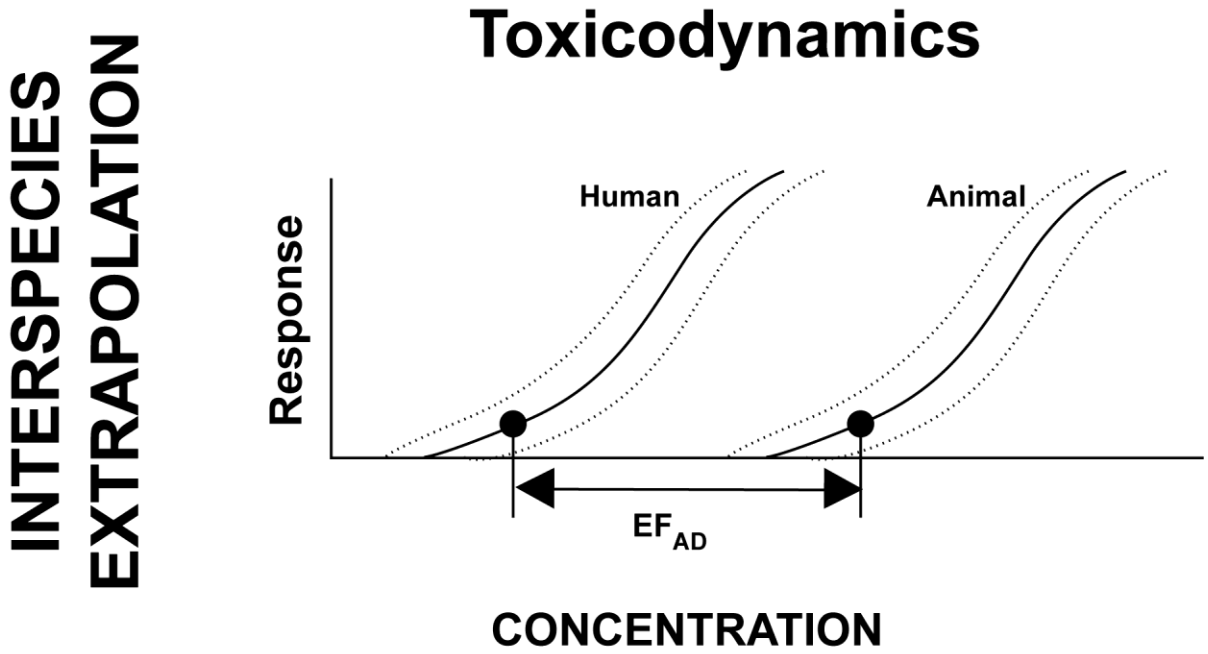

Figure 6. Interspecies toxicodynamics. The TD difference between test animals and humans is calculated from dose or concentration-response relationships most often determined in test systems or animals generally representative of the respective species. The comparison is made for doses or concentrations producing the same level of response, and comparisons are made using values representing the central tendency.

4.2.1. Considerations for EF_{AD}

4.2.1.1. *Mode of Action*

Endpoint(s) must be chosen from which to derive the EF_{AD}, and it is preferred for the endpoint(s) to be the actual critical effect or a key event in the TD pathway. For each endpoint evaluated, it must be determined whether the data are from an animal typical of the responding species/strain/sex/lifestage opposed to an animal model system that may be unusually sensitive for a particular effect. This might be important in transgenic animals and in animal models used to study specific human diseases (e.g., spontaneously hypertensive rats). Animal models and MOA(s) based on them are evaluated for human relevance.

4.2.1.2. *Target Tissues*

Where there are data from the molecular targets in both species, but the data are not from the critical target organ, the data can be used only if there is sufficient information that one tissue is an appropriate surrogate for another. For example, a target enzyme may be present in several

tissues, including blood; however, blood is easily obtained from humans, but tissue from the target organ is not. Studies may show that the chemical effect on this target enzyme in blood correlates very well and is entirely predictive of the effect in the target organ, and indeed the enzyme structure, function, and chemical affinity is the same regardless of tissue. Such information would be necessary to allow using comparisons of chemical effects in the blood enzyme in both humans and laboratory animals.

4.2.1.3. *Dose Response*

The choice of response level to use for comparison depends on many factors:

- Completeness of dose response
- Linearity of the dose response
- Understanding of the effect along the toxic pathway at that response level

The magnitude of EF_{AD} may be a function of the response level chosen for extrapolation. Confidence is increased when the response level employed for EF_{AD} calculation approximates the response level at the POD and when the ratio of doses producing the same response level in animals and humans is similar over a range of doses. When data are available to describe the full dose-response curve, evaluating the shape of the dose curves for animals and humans can provide important information. If the shapes of the curves are different, then the magnitude of EF_{AD} will depend on the response level selected. Note that concentrations causing greater magnitudes of effect may also produce nonspecific cellular changes that could confound the comparisons.

4.2.1.4. *In Vitro Data*

When using data from in vitro systems, the activity of the parent chemical and/or metabolites, as well as the extent of metabolism of the compound by the in vitro system, should be known. The in vitro assay should measure a response that can be linked to the toxic outcome, and the assays should employ the same (responding) tissues from the in vivo test animal species/strain/sex/lifestage and from humans. Experimental systems should be as closely

matched between species as possible, and the concentrations of toxicant in vitro should be compared to tissue concentrations (the dose metric) at the POD. See Section 2.2.3.2 for other general considerations.

4.2.2. Computation

4.2.2.1. *Use of TD Models*

A biologically based dose response or other TD model provides the most robust approach for evaluating interspecies TD extrapolation. The model is subject to evaluation as described previously (U.S. EPA, 2006). When available, the model can be used to inform several decisions, depending on the model. In some cases, the TD model may be used directly to perform interspecies extrapolation and to derive a Human Equivalent Concentration or Dose that includes TD considerations, thus alleviating the need for EF_{AD}. In other cases, the TD may be used to derive the EF_{AD} used.

4.2.2.2. *Use of Ratios*

When PD models are not available, EF_{AD} would be calculated as a ratio describing the relationship between the tissue concentrations producing a set response in human compared to animal tissues, preferably at, or near, the response level at the POD. For example, "x response level" may be a 10% response if ED_{10} or BMD_{10} is used. The EF_{AD} would be calculated as follows:

$$EF_{AD} = \frac{\text{Concentration}_{\text{A-}x\text{ response level}}}{\text{Concentration}_{\text{H-}x\text{ response level}}} \quad (5)$$

where

EF_{AD} = factor for interspecies extrapolation covering toxicodynamics

$\text{Concentration}_{\text{A--x response level}}$ = concentration of the agent at the tissue in the animal resulting in a *x*% response

$\text{Concentration}_{\text{H--x response level}}$ = concentration of the agent at the tissue in the human resulting in a *x*% response, where *x* is the same response value.

To evaluate the extent to which the shape of the dose-response curve varies between animals and humans and, thus, impacts the magnitude of the EF_{AD}, a range of response levels should be evaluated. The rationale and implications for choosing the point for extrapolation should also be presented.

4.2.3. Conclusions for EF_{AD}

Mathematically, the EF_{AD} will be the relationship between the concentrations or dose metric values resulting in the same level of response in both the test species and the generally representative human (often the 70-kg adult male). DDEF values may be calculated for multiple PODs and organs/effects, but the same response levels in animals and humans should be used for quantitation when possible. Confidence in the value (knowing whether the DDEF value may vary depending on the response level) is improved when EF_{AD} values developed from multiple points on the concentration-response curve are compared; the shape/slope of the curves may also influence these values. Quantitatively, EF_{AD} can be less than one if the data show humans are inherently less sensitive than animals.

A summary of all conclusions and their scientific support should be provided. Data describing the dose response of the compound in animals and humans can be specifically reiterated in this description; this will indicate the extent to which the EF_{AD} value can be generalized to doses that may be higher or lower than those used in its calculation.

4.3. INTRASPECIES TOXICODYNAMIC EXTRAPOLATION (EF_{HD})

This section describes derivation of the EF_{HD}. TD variability within the human population is calculated as the relationship between concentrations or dose metric values producing the same level of the response in the general population and in susceptible groups or individuals. From a toxicodynamic standpoint, susceptibility is based on attaining a given level of response at a lower concentration of toxicant. For this evaluation, multiple response levels, critical effects (or key events), analytical methods, or susceptible groups or individuals may be considered. No data sets were identified upon which a conclusive case study example could be developed for intraspecies toxicodynamic extrapolation.

4.3.1. Considerations for EF_{HD}

4.3.1.1. *Susceptible Groups or Individuals*

Susceptibility in the human population may be due to lifestage, health status or disease state, genetic disposition, or other factors. Considering susceptibility to more than one critical effect may require consideration of more than one life-stage; critical windows of development, and, therefore, windows of susceptibility, occur at different times for various tissues, organs, and systems. Currently, sufficient data to address susceptibility are rarely available; however, research in this area is rapidly expanding. For example, population variation, such as genetic polymorphisms, is an expanding area of study. It is anticipated that the increased availability and experience applying "omics" technologies will benefit the derivation of DDEFs, in general, and $EF_{HD,}$ in particular. A data-derived EF_{HD} is feasible, given human data are of sufficient quality; the data address aspects of the critical effect consistent with that identified from applicable human or animal studies; and the studies have been conducted in the segment(s) of individuals or the population deemed sensitive.

Ideally, data will be robust enough to enable more than point estimates in the general and susceptible groups. As discussed in more detail below, distributional analysis of response data should be conducted to identify points for use in quantitation. The relationship between the measured response and the toxicity endpoint of concern (e.g., critical effect or key event) should be described, whether determined in vivo or in vitro.

4.3.1.2. *Target Tissues*

For calculation of EF_{HD}, data for multiple responding tissues can be evaluated, and multiple DDEFs can be derived. It is particularly important to evaluate those tissues that demonstrate response at doses or concentrations near those for the critical effect.

4.3.1.3. *In Vitro Data*

Given the constraints on generation of human response data in vivo, in vitro studies offer an appealing alternative. Samples selected for in vitro investigation should represent the general human population as well as those groups or individuals thought or demonstrated to be susceptible. See Section 2.2.3.2 for other general considerations.

4.3.2. Computation

For TD extrapolation, the goal is to determine the difference between humans on the basis of concentration producing the same response level. For quantitation, data on the critical response(s) are derived from a population that includes susceptible groups or individuals. Because the data available to define potentially susceptible groups or individuals could be viewed in different ways, a statistical analysis may be helpful to determine distribution type (see Figure 7):

- A unimodal distribution where the potentially susceptible group(s) represent the tail of the distribution because they cannot be separated from the general population.
- A bimodal (or multimodal) distribution where the group(s) can be readily identified.

Documenting critical response data, assumptions made, and the distribution selected will serve as the basis for quantitation.

4.3.2.1. *Use of TD Models*

A biologically based dose response or other TD model provides the most robust approach for evaluating intraspecies TD extrapolation. When sufficient data are available, these TD models can be structured and exercised to include differences in mode-of-action components that may be lifestage-dependent or influenced by other potentially susceptibility-inducing conditions such as genetic polymorphisms. Specific to EF_{HD}, it is critical that the model parameter reflecting the underlying cause of susceptibility in a group be well documented. When an appropriate model is available, it can be used in different ways depending on the model. In some cases, the TD model may directly account for within human variation and/or include data from the sensitive group, thus eliminating the need for EF_{HD}. In other cases, the TD may be used to derive EF_{HD}.

4.3.2.2. *Use of Ratios*

When TD models are not available and there are groups or individuals that can be identified as sensitive, then the EF_{HD} may be defined as the ratio between the concentrations

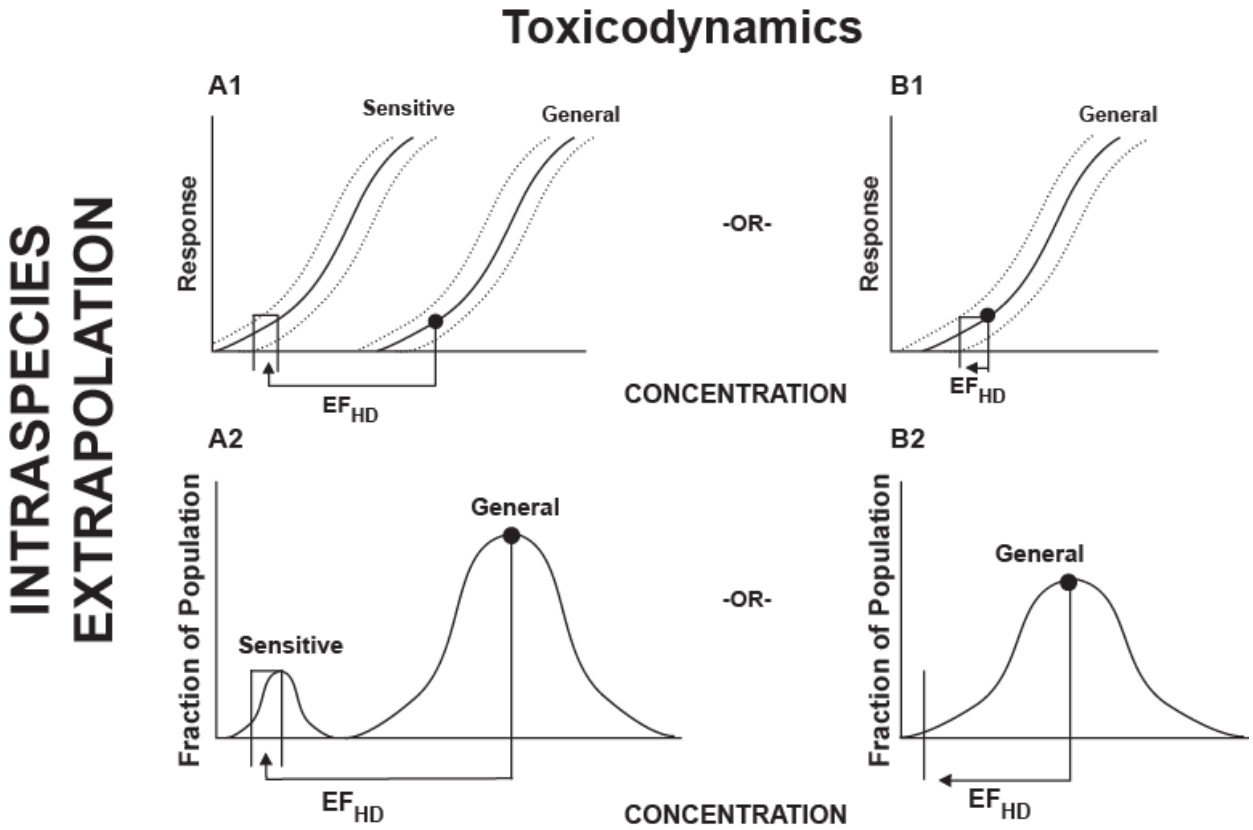

Figure 7. Intraspecies toxicodynamics. DDEF values for TD are defined by ratios of concentrations producing the *same level of response* in the general population and some defined percentile (e.g., 1st, 2.5th, 5th, etc.) for the distribution representing sensitive individuals. When a specific group of the population can be identified as potentially susceptible, TD data from that group can be compared to the general population (Panels A1 and A2). Panel A1 presents a dose-response curve (cumulative distribution plot) for both populations that demonstrates the central tendency (solid line) and confidence bounds, or bounds of variability (dashed lines), for data obtained from the general population and from an identifiable sensitive group. In this example, the level of response (Y-axis) has been selected (e.g., 10%-response level), and the concentrations producing this level of response in the general and sensitive populations/groups are obtained from the X-axis. Panel A2 is derived from the same data used for Panel A1, but it presents the distribution of concentrations producing the defined level of response only; no other dose-response data are carried over into Panel A2. Alternately, when potentially susceptible individuals represent a small percentage of the general population (Panels B1 and B2), a slightly different analysis is conducted. In this case, EF_{HD} should be determined as the ratio of the concentrations producing the same level of response (1) at a measure of the central tendency in the population to (2) the concentration producing the response level at a percentile of the general population considered sensitive (e.g., 1st, 2.5th, 5th percentile). Panel B1 demonstrates this comparison using the concept of confidence bounds on the dose-response relationship, and Panel B2 demonstrates the distribution of concentrations producing the response, only at the response level chosen for comparison (e.g., the 10%-response level).

producing the same level of response in the general population and a lower percentile in the sensitive group (see Figure 7) using Equation 6:

$$EF_{HD} = \frac{\text{Concentration}_{gen}}{\text{Concentration}_{sens}} \quad (6)$$

where

EF_{HD} = factor for intraspecies extrapolation covering toxicodynamics

$\text{Concentration}_{gen}$ = concentration producing the in response the general human population

$\text{Concentration}_{sens}$ = concentration producing the response at a percentile of interest for the sensitive group.

When sensitivity among the population exhibits a unimodal distribution, the EF_{HD} is the ratio of the concentration that elicits a level of response at the central tendency of the distribution to the concentration that elicits the same level of response in sensitive individuals (e.g., 5^{th}, 2.5^{th}, and 1^{st} percentiles of the distribution; sensitive individuals will respond at lower concentrations). It is important to define and justify the point(s) in the distribution representing sensitive groups or individuals.

When sensitivity among the population exhibits a bimodal (or multimodal) distribution, the DDEF is determined in a similar manner, using the concentrations (e.g., 5^{th}, 2.5^{th}, and 1^{st} percentiles of the concentration distribution) that elicit the specific level of response in the sensitive individuals for the most susceptible group(s). The values selected to describe the potentially sensitive group(s) or individuals are defined and presented at varying levels. The selection of the response level and the percentile of the distribution used to describe the potentially sensitive group(s) or individual(s) is an important issues. This is a situation where the communication between risk assessment and risk management is essential.

4.3.3. Conclusions for EF_{HD}

A biologically based dose response or other TD model provides the most robust approach for evaluating intraspecies TD extrapolation. When using empirical ratios, the EF_{HD} will be the

ratio of the concentration producing the specified level of response in the general human population to the concentration producing the same level of response in susceptible groups or individuals. Increased confidence in the EF_{HD} is developed when the concentration used for the comparison of responses is compared to doses or concentrations at the POD. Quantitatively, EF_{HD} cannot be less than one.

The risk assessor describes all choices and their rationales, including the use of multiple response levels, critical effects (or key events), analytical methods, or data from susceptible groups or individuals. The conclusions include a clearly worded description of the mathematical method(s) employed and a presentation of the relationship between the measured response and toxicity (i.e., critical effects or key events). This description should clearly identify and provide the justification for available data and points in the distribution(s) representing sensitive individuals. Attention should be paid to characterizing the distribution type employed for analysis; uncertainty in the choice of distribution type can be reduced by presenting DDEF values resulting from multiple distribution types.

5. FINAL STEPS

The composite DDEF is calculated after the appropriate DDEF values for inter- and intraspecies differences in TK and TD have been derived. The composite factor is calculated by multiplying the specific factors (default and/or DDEFs), as shown in Equation 7. This is entirely analogous to calculating composite UFs when using the 10× defaults for UF_A and UF_H. The composite DDEF may be less or greater than 100.

$$CF = EF_{AK} \times EF_{AD} \times EF_{HK} \times EF_{HD} \quad (7)$$

where

CF = composite uncertainty factor

EF_{AK} = factor for interspecies extrapolation covering toxicokinetics

EF_{AD} = factor for interspecies extrapolation covering toxicodynamics

EF_{HK} = factor for intraspecies extrapolation covering toxicokinetics

EF_{HD} = factor for intraspecies extrapolation covering toxicodynamics

In practice, data may only be available to develop a DDEF for one component of extrapolation or another (e.g., data for EF_{AK} but not EF_{AD}). In these cases, the remaining extrapolation is done by an appropriate default procedure. As such, DDEFs and defaults (i.e., UFs) are used in combination. Often this default will be a 3× UF—as described in the existing RfC methodology and the ¾ body-weight procedure (U.S. EPA, 2011, 1994). When data are not available to develop DDEFs for either component of interspecies or intraspecies extrapolation, the 10× default value for the uncertainty factor is applied.

Finally, the composite factor provides the total magnitude of the factor. The values derived for each of the components and the resulting extrapolations should be clearly reported and characterized. The relationship of each of these doses or concentrations to both the POD and to doses or concentrations likely attained from environmental exposures should be presented.

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