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External Peer Review Draft
October 2008

Application of Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation

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The technical assistance of Ms Bette Zwayer is gratefully acknowledged.

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LIST OF ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, Elimination; toxicokinetics
AUC	Area under the curve
AUC _{%ile}	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
CSAF	Chemical specific adjustment factors
CYP	Cytochrome P450
DDEF	Data-derived uncertainty 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
UF _{AD}	Toxicodynamic component of interspecies uncertainty factor
UF _{AK}	Toxicokinetic component of interspecies uncertainty factor
UF _H	Intraspecies Uncertainty Factor

LIST OF ABBREVIATIONS cont.

UF _{HD}	Toxicodynamic component of intraspecies uncertainty factor
UF _{HK}	Toxicokinetic component of intraspecies uncertainty factor
V _{max}	Theoretical maximal initial velocity
WHO	World Health Organization

PREFACE

This document is intended to help U.S. Environmental Protection Agency (U.S. EPA) risk assessors take a data-based approach to non-linear low dose extrapolation. It describes data recommendations and methods or procedures to calculate data-derived extrapolation factors (DDEF) for chemicals for interspecies and intraspecies extrapolation. The U.S. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (or "*Cancer Guidelines*") (U.S. EPA, 2005) describes the U.S. 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 U.S. EPA uses default assumptions or processes in order to continue with the risk assessment. Under this practice the U.S. EPA invokes defaults only after the data are determined not to be usable at a particular point in the assessment. Both the *Cancer Guidelines* and the U.S. 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 DDEF draft document, *Application of Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intra-species Extrapolation* provides such a description.

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 low-dose extrapolation. It presents some issues and describes some points to consider when evaluating data for the purpose of inter- and intraspecies extrapolation factor development. This will contribute to consistency in the U.S. EPA processes and decisions.

1. INTRODUCTION

1.1. BACKGROUND

Risk assessment is the U.S. Environmental Protection Agency (U.S. EPA)'s key process for deriving scientific information for making sound decisions on managing risks to human health and the environment (U.S. EPA, 2004). Thus, risk assessors aim to develop estimates of risk that best reflect the true risk to the human population or the environment based on state-of-the-science methods. No risk assessment can reflect this 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 U.S. 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 (DDEF).

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

- *Risk Characterization Handbook* (U.S. EPA, 2000)
- *Guiding Principles for Monte Carlo Analysis*
- the 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 U.S. 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 U.S. EPA's use of defaults as a reasonable way to deal with uncertainty. That report stated that the U.S. EPA should have principles for choosing default options and for judging when and how to depart from them.

Since then, the U.S. EPA now initiates the process of choosing a method for developing uncertainty factors by evaluating the available data—default values are invoked 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 *Cancer Guidelines* 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*

1 needed to address uncertainty or the absence of critical information.” Thus, while risk assessors
2 have generally tried to make maximum use of available data, the shift away from standard
3 default assumptions was codified as U.S. EPA science policy with the publication of the 2005
4 *Cancer Guidelines*. Evaluating the available data will improve the scientific basis of risk
5 assessments when data are sufficient for refining uncertainty factors (UFs). In cases where data
6 are not sufficient, hazard and risk characterizations will be improved and data needs can be noted
7 and potentially filled in the future (Murray and Andersen, 2001; Meek, 2001; Meek et al., 2001;
8 Bogdanffy et al., 2001).

10 1.2. PURPOSE AND SCOPE

11
12 The United States and international efforts have improved the scientific basis for human
13 health risk assessments by increasing the use of mechanistic and kinetic data. For example, the
14 U.S. EPA’s 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) emphasize the
15 use of mode of action (MOA) information in characterizing potential health effects of exposure
16 to environmental agents. International efforts including those by the International Life Science
17 Institute (ILSI) and the World Health Organization (WHO)’s International Programme on
18 Chemical Safety (IPCS), have developed frameworks for evaluating animal data to determine the
19 human relevance of described MOAs (Seed et al., 2005; Sonnich-Mullin et al., 2001; Boobis et
20 al., 2008). These documents guide the qualitative and quantitative evaluation of the relevance of
21 a particular animal model of action in humans and discuss the use of *in vivo* and *in vitro* data
22 when considering animal to human extrapolation. The *Guidelines for Carcinogen Risk*
23 *Assessment* (U.S. EPA, 2005), and other documents like IPCS’s chemical specific adjustment
24 factors (CSAF) guidance (IPCS, 2005), the *Methods for Derivation of Inhalation Reference*
25 *Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), and *An Examination*
26 *of EPA Risk Assessment Principles and Practices: Staff Paper Prepared for the U.S. EPA by*
27 *Members of the Risk Assessment Task Force* (U.S. EPA, 2004) also encourage the use of
28 sophisticated models like physiologically based pharmacokinetic (PBPK) and biologically based
29 dose response (BBDR) models in interspecies extrapolation.

30
31 This document deals specifically with the use of data-derived factors in the calculation of
32 non-linear low dose estimates, or safety assessments. The goal of DDEFs is to maximize the use
33 of available data and improve the scientific support for a risk assessment. The processes
34 described herein have benefited from the continuing discussion in the scientific community on
35 ways to replace the 10-fold Uncertainty Factors (10× UFs) that have historically been used in
36 deriving safety assessments such as reference doses (RfD), minimal risk levels and acceptable
37 daily intakes. WHO’s IPCS guidance for deriving CSAFs was finalized in 2005. This CSAF
38 guidance describes approaches for use of kinetic and mechanistic data to refine interspecies and
39 intraspecies extrapolation factors. The IPCS guidance is largely based on analyses by Renwick
40 (1993) and Renwick and Lazarus (1998), which describe the use of toxicokinetic and
41 toxicodynamic data as a means of replacing the traditional 10× for human sensitivity and
42 experimental animal-to-human extrapolation. This data-derived approach assigns values for
43 toxicokinetic and toxicodynamic differences as replacements for each traditional 10× UF.
44 Important distinctions between IPCS (2005) and the present U.S. EPA guidance are that IPCS
45 restricts toxicokinetic evaluations to the central compartment, disallowing local tissue
46 metabolism to be quantified as part of the toxicokinetic processes; division of the animal to

1 human extrapolation unevenly, attributing a greater fraction of default uncertainty to TK than to
2 TD; and a general level of depth.

3
4 The current document describes the U.S. EPA's approach to calculating extrapolation
5 values based on data; these are called *data-derived extrapolation factors (DDEF)*. DDEFs are
6 similar in concept to IPCS/WHO's CSAFs in that the standard extrapolation factors are separated
7 into toxicokinetic (TK) and toxicodynamic (TD) components, and kinetic and mechanistic data
8 are used to derive refined interspecies or intraspecies extrapolation factor(s). Conceptually,
9 DDEFs (and CSAFs) may not be limited to a specific chemical but may also apply to chemicals
10 with common structural characteristics, common MOA or common toxicokinetic characteristics
11 or determinants. An appendix to this document contains case study examples taken from the
12 Integrated Risk Information System (IRIS) and from Program Office records. These case studies
13 present the application of principles contained in this document to data and modeling studies for
14 actual chemicals and should serve as instructional aides.

15
16 Topics most relevant to the derivation and use of DDEFs are the focus of this document.
17 Thus, there are concepts beyond the scope of this guidance that are not discussed in detail here:
18 approaches for selecting critical effects; establishing key events in an MOA analysis;¹ deriving
19 points of departure; performing benchmark dose analysis; and developing and evaluating PBPK
20 and BBDR models. In addition, this document deals only with DDEF for the areas of
21 interspecies and intraspecies extrapolation; there is no discussion of factors that have been used
22 for other areas of uncertainty or variability (e.g., duration, database deficiencies and lack of a no-
23 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 intra-species 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 U.S. EPA (the Agency) derivation of reference concentrations (RfC) and RfDs (the predominant U.S. EPA nonlinear approaches) recognize steps for interspecies 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, 1993, 1994, 2002a, 2006a). This document describes an approach to performing interspecies 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, a class of chemicals with shared chemical or toxicological properties and for a group of chemicals which 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 U.S. EPA currently recognizes a hierarchy of approaches ranging from the preferred approach using PBPK modeling (U.S. EPA, 1994, 2006b) 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

For the oral exposure route, the default approach for interspecies extrapolation involves scaling the applied dosing, according to body weight, to the $\frac{3}{4}$ power and a residual uncertainty factor (U.S. EPA, 2006a). 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 suggest that the interspecies uncertainty factor might be evenly divided between TK and TD components. These values are one-half 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 one-half 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 default (oral or inhalation route) has a default value of 3, which may be modified based on available data (U.S. EPA, 1994, 2006a). 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 $\frac{3}{4}$ body weight scaling.

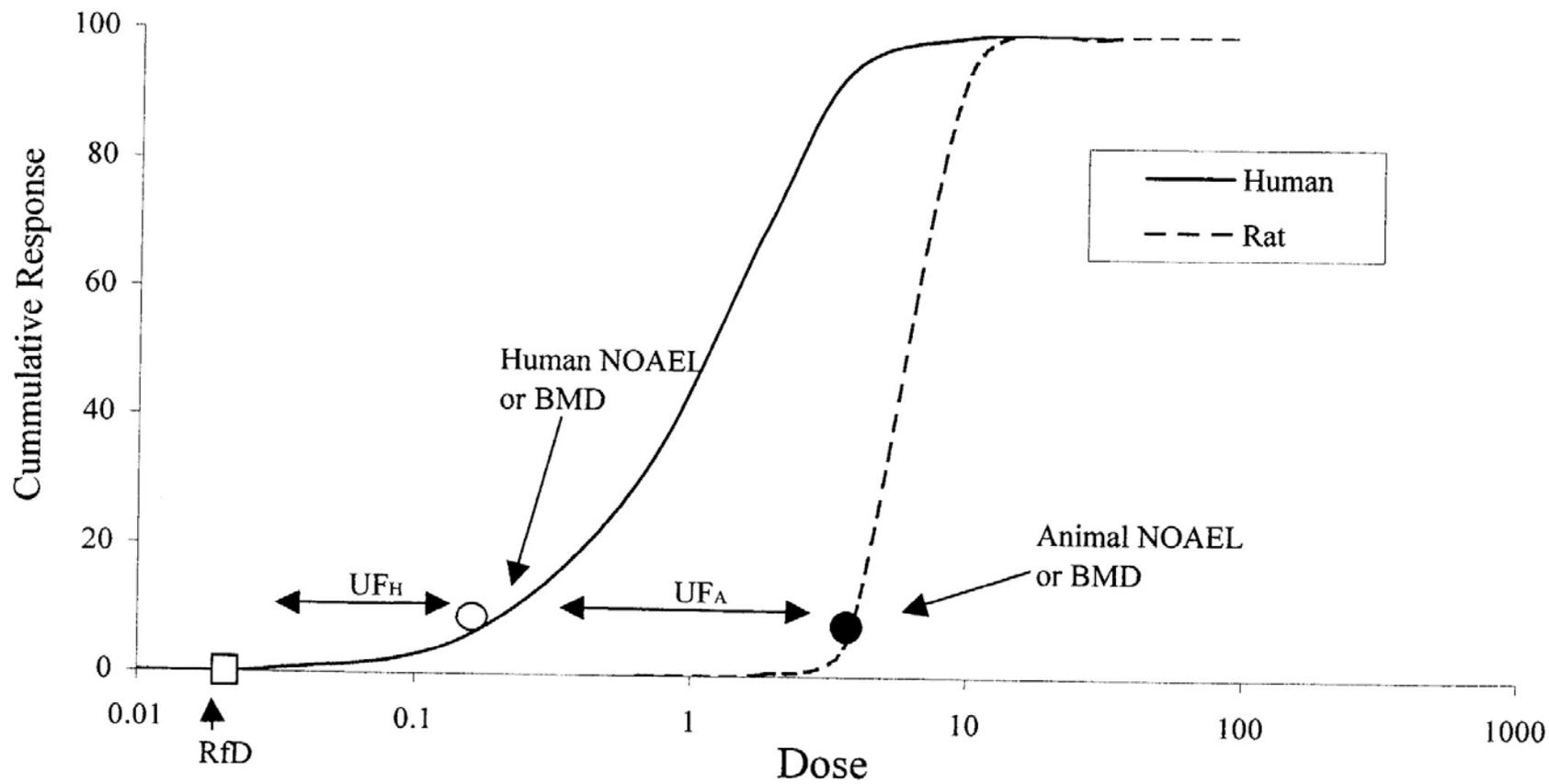


FIGURE 1

Derivation of RfDs/RfCs Using Uncertainty Factors

1
2
3
4
5

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 calculating relatively simple ratios using TK or TD data describing critical factors in inter- or intraspecies extrapolation. The following text describes these approaches. Figure 2 is a flowchart depicting the decision process used in deriving DDEFs.

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). The common factor among these models is that they relate external dose or observed response to some measure of internal dose or response.

TK modeling is the process of developing a mathematical description of ADME in a living organism. Two common types of model are (1) data-based classical non-compartmental 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 multi-compartment 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 comprised 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

Data-Derived Extrapolation Factors

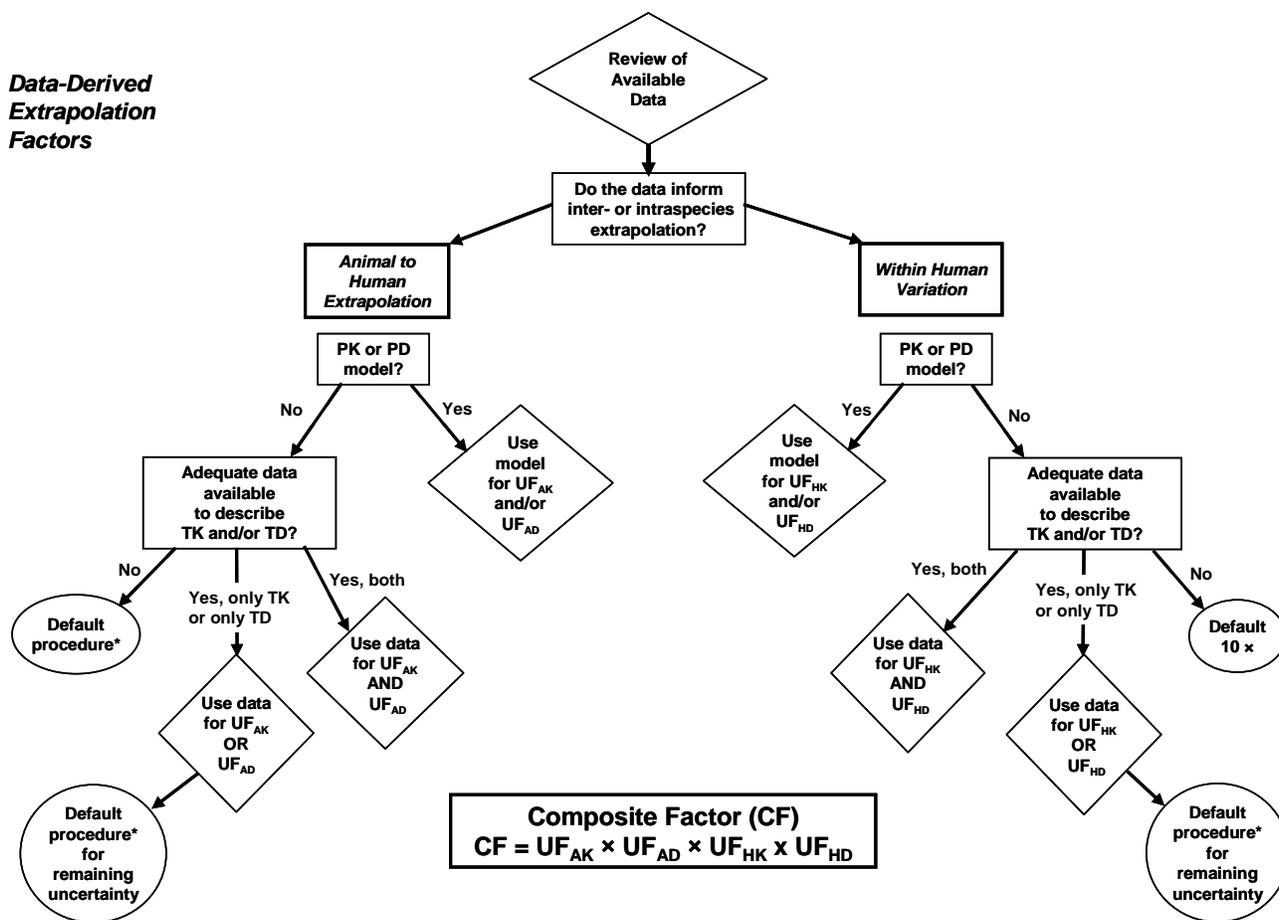


FIGURE 2

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 (UF_{AK} , UF_{AD}) and interspecies extrapolation (UF_{HK} , UF_{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 $\frac{3}{4}$ 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 3X 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 uncertainty factor components.

1 in both humans and laboratory animals (usually using *in vitro* techniques); these chemical-
2 specific parameters include tissue solubility (i.e., partition coefficients), binding and metabolism.
3 These models are used to simulate the relationship between applied dose and internal dose. They
4 are more data-intensive to develop compared to classical compartmental models, but they are
5 advantageous because they can be used for extrapolation (i.e., across dose range, among animal
6 species, between routes of exposure and across exposure scenarios) (Krishnan and Andersen,
7 1994; U.S. EPA, 2006b).

8
9 TD models can be developed when there are sufficient data to both ascertain the MOA
10 and to quantitatively support model parameters that represent rates and other quantities
11 associated with key precursor events in the MOA. A BBDR model describes biological
12 processes at the cellular and molecular level in such a way as to link target tissue dose with
13 adverse effect; in practice BBDR models are often described as a combined TK/TD model.
14 These models may be used for extrapolation.

15 16 **2.2.1.2. Use of Ratios to Calculate DDEF**

17
18 In the absence of sufficient data to develop a robust TK or TD model, the risk assessor
19 need not necessarily use default 10× UFs. DDEFs can be calculated as ratios using data from
20 key studies evaluating TK or TD profiles or properties of a particular chemical. Example
21 equations for calculating DDEFs are provided in Table 1 and described in more detail in Sections
22 3 (TK) and 4 (TD).

23
24 In general, *interspecies* extrapolation involves calculating a ratio of human data for a
25 kinetic or dynamic parameter to animal data for a kinetic or dynamic parameter. Similarly for
26 *intraspecies* extrapolation, a ratio is calculated using data from the sensitive population and that
27 for the general, or average, population. Data to derive the TK factors may come from *in vivo* or
28 *in vitro* studies. For TD, in general, interspecies extrapolation may come from *in vivo studies* but
29 will often be accomplished with *in vitro* data in a relevant tissue. When data on toxic effects are
30 available in humans, these data may be used directly for the point of departure (POD)
31 development obviating the need for the interspecies extrapolation. Likewise they can be used to
32 inform an interspecies factor when the POD is derived from animals.

33
34 For DDEFs involving interspecies extrapolation, it is preferred that the ratio be based on
35 data at or near the POD. When sufficient data are available, DDEF values should be calculated
36 for a range of doses near the POD because the shape of the dose-response curve can vary among
37 species. Metabolism and kinetic properties can vary across doses, particularly in the higher dose
38 ranges; thus using estimates at or near the POD helps avoid introducing significant uncertainty in
39 the DDEF estimate caused by nonlinearity in kinetic properties. Evaluating a range of PODs
40 takes into account the variability of the DDEFs based on the POD selected. The interspecies
41 DDEF should be derived using an estimate of central tendency, such as the mean, median or
42 mode, depending on the characteristics of the data. It is, however, important to evaluate
43 variability in the DDEF. Thus, it is recommended that, to the extent possible, the hazard and risk
44 characterizations reflect the upper and lower confidence bounds on the DDEF.

1

<p style="text-align: center;">TABLE 1</p> <p style="text-align: center;">Example Equations Used to Derive DDEFs</p>		
Extrapolation	Toxicokinetic (Section 3)	Toxicodynamic (Section 4)
Animal to Human (Interspecies)	$UF_{AK} = \frac{Dose_A}{Dose_H}$	$UF_{AD} = \frac{Concentration_A}{Concentration_H}$
Within Human (Intraspecies)	$UF_{HK} = \frac{AUC_{gen}}{AUC_{\%tile}}$	$UF_{HD} = \frac{Concentration_{gen}}{Concentration_{sens \%tile}}$

- 2
- 3 UF_{AK} = uncertainty factor for interspecies extrapolation covering toxicokinetics
- 4 $Dose_A$ = administered or external dose to the animal
- 5 $Dose_H$ = administered or external dose to the human
- 6 UF_{AD} = uncertainty factor for interspecies extrapolation covering toxicodynamics
- 7 $Concentration_A$ = concentration of the agent at the tissue in the animal
- 8 $Concentration_H$ = concentration of the agent at the tissue in the human
- 9 UF_{HK} = uncertainty factor for intraspecies extrapolation covering toxicokinetics
- 10 AUC_{gen} = area under the curve at a measure of central tendency in the general human population
- 11 $AUC_{\%tile}$ = area under the curve at a percentile of interest in the human population
- 12 UF_{HD} = uncertainty factor for intraspecies extrapolation covering toxicodynamics
- 13 $Concentration_{gen}$ = concentration at a measure of central tendency in the general human population
- 14 $Concentration_{sens \%tile}$ = concentration at a percentile of interest in the human population

1 By contrast to interspecies extrapolation, when calculating intraspecies DDEFs, the ratio
2 involves consideration of a measure of central tendency of the general population and lower
3 percentiles of interest (e.g., 1st, 2.5th or 5th) to represent the sensitive populations. As the needs
4 of risk managers and decision makers vary, it is recommended that a range of percentiles be
5 evaluated and reported in the hazard and risk characterizations.
6

7 Toxicokinetic ratios (for either interspecies or intraspecies extrapolation) are based upon
8 the relevant dose metric, such as area under the curve (AUC) and the maximum concentration
9 (C_{\max}).³ Other metrics (e.g., AUC above a threshold) may be used if supported by the data or if
10 relevant for a particular chemical or MOA. For toxicants which bind covalently or cause
11 irreversible damage, especially as a consequence of subchronic or chronic exposure, an
12 integrated measure of dose over time such as AUC is generally used (O'Flaherty, 1989). In the
13 case of effects occurring as a consequence of acute exposure, C_{\max} may be more appropriate
14 (Boyes et al., 2005; Barton, 2005). When data on chemical-specific AUC, C_{\max} or clearance (Cl)
15 are not available, a chemical-related physiological parameter (e.g., renal glomerular filtration
16 rate) that is critical to the onset of toxicity or to the MOA may be used.
17

18 As Table 1 indicates there are generally 4 DDEFs that can be calculated, given sufficient
19 information. Two are for extrapolation from animal data to humans: UF_{AK} is calculated to
20 account for TK variability, while UF_{AD} deals with TD variability. Likewise there are two factors
21 dealing with variability within the human population: UF_{HK} for TK and UF_{HD} for TD. Table 1
22 provides example equations for calculating these DDEFs. Section 3 describes specifics for TK
23 factors for interspecies (Section 3.2) and intraspecies extrapolation (Section 3.3). Section 4
24 describes TD factors for both animal to human (Section 4.2) and within human extrapolation
25 (Section 4.3). Section 5 describes how to combine the UF_{AK} , UF_{AD} , UF_{HK} and UF_{HD} into the
26 composite UF.
27

28 The overall goal of DDEFs is to maximize the use of available data and improve the
29 overall scientific support for a risk assessment. Figure 2 provides a flowchart of the decision
30 process for extrapolation used in deriving DDEFs. As shown in the figure, inter- and
31 intraspecies extrapolation can be accomplished using a combination of TK or TD models,
32 DDEFs derived from ratios, and/or use of defaults. As described in more detail in Sections 3 and
33 4, it is important for the hazard and/or risk characterizations to include thorough and transparent
34 discussions of methods and data used to support extrapolation approaches.
35

36 2.2.2. Qualitative Considerations

37

38 Although in some cases there may not be sufficient data for a quantitative estimate of a
39 DDEF, there may still be information to support a UF different from the default. For example,
40 there may be qualitative evidence that a MOA identified in animals is not relevant to humans. A
41 framework developed by ILSI for evaluating the relevance of an animal MOA can be found in
42 Seed et al. (2005), Meek et al. (2003) and Boobis et al. (2008). The human relevance framework
43 provides a transparent and logical thought process by which animal and human MOA data can be
44 evaluated on both a qualitative and quantitative basis. In these cases where only qualitative data

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.

1 are available, a thorough weight-of-evidence analysis should be considered with the hazard
2 and/or risk characterization to discuss the derivation of the DDEF along with associated
3 uncertainties in the available database.

4 5 **2.2.3. Information Quality**

6
7 Critical evaluation of all data used to support the development of DDEFs is necessary.
8 This includes data used to provide qualitative support for the MOA and choice of dose metric, as
9 well as data used in the quantitative derivation of the DDEF itself. Supporting studies can be
10 evaluated using criteria set forth in various U.S. EPA guidance documents, including the recently
11 published Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), as well as earlier
12 guidelines specific to neurotoxic, reproductive and developmental endpoints (U.S. EPA, 1998,
13 1996, 1991). In addition, the general principles outlined in the U.S. EPA information quality
14 guidelines are applicable in the critical evaluation of data used to support DDEF development
15 (U.S. EPA, 2002a). The remainder of this section highlights some areas of special emphasis that
16 are particularly relevant to the DDEF derivation process including MOA, uncertainty and
17 variability and dealing with multiple responding organs or tissues.

18
19 Use of secondary data sources is one particular area of concern. Examples of secondary
20 data sources include compilations of pharmacokinetic parameters (e.g., Brown et al., 1997) and
21 studies cited and summarized in toxicity profiles and review articles. In general, for principle
22 and supporting studies used directly in DDEF derivation, review of the original literature is
23 necessary. In the case of critical assumptions and data, contradictory results from different
24 studies are best resolved by review of the original publications.

25
26 Quantitative TK and TD data used in the DDEF derivation process requires particular
27 attention to appropriateness of the study design, the analytical methodology used and the
28 statistical analysis of the data. Consideration of appropriate study design extends beyond simply
29 verifying that the methods used were adequate for the goals of the study; it also encompasses
30 consideration of the relevancy of the animal species or *in vitro* test system to evaluate MOA.
31 Relevance can be assessed in both qualitative and quantitative terms. For example, if there is a
32 lack of species concordance (i.e., a particular TK or TD process does not occur in humans) or
33 effects occur only under physiologically unrealistic conditions or not in the tissue evaluated, then
34 its relevancy is questionable and uncertain. Criteria used in arriving at such a determination have
35 been published for both the more general case (Seed et al., 2005) and particular endpoints
36 including various forms of rodent cancer (Proctor et al., 2007; Maronpot et al., 2004). Particular
37 considerations relevant to the use of *in vitro* data are discussed below. Another important factor
38 in terms of relevancy is consideration of whether the TK or TD response represents a uniquely
39 susceptible tissue, process or population. This is a critical determinant in evaluating the use of
40 data to describe intraspecies variability.

41
42 Analytical methodologies used for chemical identification and quantification are a critical
43 factor in data quality. Thus, the analytical technique for compound detection needs to be robust,
44 applicable to the biological media sampled and statistically reliable (e.g., sensitivity, specificity;
45 and recovery of measured analyte). In addition, the limit of detection or quantification of the
46 analytical technique must be low enough to permit reliable measurements at doses and time

1 points applicable to the experiment in which it was used. The appropriateness of statistical
2 methodologies used is critical to both TK and TD endpoints. This includes consideration of
3 sample size, power to detect a specific level of effect (difference from control or background)
4 and ability to detect between-group differences.
5

6 Quantitative kinetic and dynamic models can be critical tools for the derivation of
7 DDEFs. These models can range from relatively simple compartmental or statistical models to
8 more complex physiologically based models. Two critical considerations in model use are
9 evaluation appropriateness for the proposed range of extrapolation and evaluation of the model
10 itself. Consideration of the range of extrapolation encompasses the issue of whether the model
11 describes the relevant data sufficiently within the range of the data, especially surrounding any
12 inflection point or POD. Evaluation of the model itself includes assessment of the
13 appropriateness of parameter values used as model input, biological assumptions underlying the
14 model and the availability of the model code for testing and evaluation against independent data.
15 Sensitivity analysis can be used to identify the most sensitive parameters for relevant dose
16 metrics in order to assist in evaluating whether the model is appropriate for the proposed use
17 (Clark et al., 2004; U.S. EPA, 2001, 2006b).
18

19 **2.2.4. Additional Considerations**

20 **2.2.4.1. Mode of Action**

21 Information on MOA can greatly enhance DDEF derivation, even when a complete
22 explication of mechanism is not available. In the 2005 *Cancer Guidelines*, the U.S. EPA
23 describes MOA evaluation as the critical information that defines the conditions under which a
24 toxicant causes its effect, the relevance of animal data for hazard identification and the most
25 appropriate approach to low-dose extrapolation. The *Cancer Guidelines* also present a
26 framework for evaluating data in support of MOA determination. Major components of this
27 framework include description of the hypothesized MOA and discussion of the experimental
28 support for the hypothesized MOA based on modified Hill criteria (U.S. EPA, 2005) for
29 demonstrating associations in human studies.
30
31

32 MOA is defined as a sequence of key events and processes, starting with the interaction
33 of an agent with a cell, proceeding through operational and anatomical changes, and resulting in
34 toxicity. A key event is an empirically observable precursor step that is itself a necessary
35 element of the MOA or is a biologically based marker for such an element. MOA is contrasted
36 with “mechanism of action,” which implies a more detailed understanding and description of
37 events, often at the molecular level, than is meant by MOA (U.S. EPA, 2005).
38

39 DDEFs for both TK and TD are endpoint-driven; that is, considered in the context of the
40 toxic endpoints most relevant for purposes of the risk assessment. Understanding MOA for the
41 agent(s) of interest helps to ensure that the TK or TD parameter used to derive the DDEF will be
42 robust scientifically. The key events in MOA are likely to identify important metabolite(s) and
43 potential species differences. Moreover, data on key events may be used directly to estimate the
44 UF_{AK} or the UF_{AD} .
45

1 2.2.4.2. *Use of In Vitro Data*

2
3 *In vitro* assays play an important role in defining DDEFs; however, care must be taken to
4 avoid taking isolated findings out of context. Consideration of interspecies differences in ADME
5 is essential because the dose to target tissue in any given exposure scenario is a balance among
6 multiple and competing ADME processes. Thus, *in vitro* data should not be used for quantitative
7 purposes unless interpreted in the context of the intact system. Among the questions to be
8 considered when applying *in vitro* data to DDEFs are:

- 9
- 10 • Was the toxicologically active form of the agent studied?
 - 11 • How directly was the measured response linked to the toxic effect?
 - 12 • Are the biological samples used in the assays derived from equivalent organs, tissues, cell
13 types, age, stage of development, and sex of the animals/humans in which the target
14 organ toxicity was identified?
 - 15 • What is the range of variability (e.g., diverse human populations and lifestages) that the
16 biological materials cover?⁴
 - 17 • If the effect occurs or can be measured in several tissues, is the studied tissue or tissue
18 preparation an appropriate surrogate? --OR-- In situations where the effect is not
19 localized, is the effect consistent across tissues?
 - 20 • Does the design of the study allow for statistically valid comparisons based on such
21 factors as replication and sample size?
 - 22 • Was chemical uptake considered when the chemical was applied to the samples so as to
23 give comparable intracellular concentrations across tissues, and similar tissues across
24 species?
 - 25 • Do the concentrations in the *in vitro* studies allow for comparison with *in vivo*
26 conditions?

27
28 All of these issues affect the utility of applying *in vitro* data for risk assessment: a clear
29 discussion of these points helps to clarify the appropriateness of the information used for
30 deriving DDEFs.

31 32 2.2.4.3. *Uncertainty and Variability*

33
34 The application of the inter- and intraspecies UFs attempts to account for both the
35 uncertainty (lack of knowledge) and the variability (true heterogeneity) in the data available (see
36 Textbox 1, U.S. EPA, 2002a). The DDEFs described in this document evaluate variability
37 within the data. Evaluation of the sources and magnitude of uncertainty is appropriate (U.S.

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

1 EPA, 1997a,b, 2001, 2005). Quantitative uncertainty analyses may be undertaken but are not
2 presented in this document. When quantitative approaches are not feasible, qualitative
3 uncertainty analyses may be developed. As is consistent with the Cancer Guidelines: “a default
4 option may be invoked if needed to address uncertainty or the absence of critical information.”
5

6 **2.2.4.4. Multiple Critical Effects**

7
8 For some toxicants multiple critical
9 effects may be identified during hazard
10 identification. In some cases, these effects
11 may be the result of a single MOA.
12 However, for others, the critical effects may
13 have different or unknown MOAs. It is
14 possible that the uncertainty and/or
15 variability associated with the TK and/or TD
16 of each effect may differ, resulting in
17 different DDEFs. The results generated for
18 the multiple responding tissues/organs,
19 particularly if multiple MOA are operational
20 or MOA is unknown, should be presented
21 for comparison (for example, in a table that
22 is accompanied by a discussion of the
23 methods used). Unless there is scientific
24 support for doing so, it is important not to
25 mix DDEFs derived for one tissue or one MOA with DDEFs derived from a different tissue. For
26 example, DDEF values for kidney effects may not apply to liver effects.
27

28 **2.2.4.5. Screening Level vs. Refined Risk Assessments**

29
30 Extrapolation is most scientifically robust when data are first evaluated prior to the use of
31 defaults. However, with a multitude of types of data, analyses and risk assessments, as well as
32 the diversity of needs of decision makers, it is neither possible nor desirable to specify step-by-
33 step criteria for decisions to invoke a default option. Some risk assessments may be limited by
34 time or resource constraints. Other risk assessments may provide only screening level
35 evaluations. In these cases, the risk assessment may be more likely to resort to one or more
36 default assumption. On the other hand, risk assessments used to support significant risk
37 management decisions will often benefit from a more comprehensive assessment.

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 is 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 (2002).

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. This section provides a discussion of factors common to derivation of both interspecies 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 (UF_{AK}); differences in susceptibility within the human population are used for the intraspecies extrapolation (UF_{HK}). Thus the factor UF_{AK} accounts for extrapolation from laboratory animals to the general human population. The UF_{HK} factor accounts for the variation in the dose/exposure-response relationship between the general human and susceptible 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).

The TK portion of each UF (UF_{AK} , UF_{HK}) is combined with the corresponding TD factors to assemble the composite UF (see Section 5). Where the data are not sufficient to derive a DDEF for TK, other approaches can be considered for UF_{AK} or UF_{HK} . For example, the RfC approach (U.S. EPA, 1994) when evaluating inhalation data or $\frac{3}{4}$ body weight scaling, or a default as described in Figure 2.

Important questions to address for UF 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?
- Is the metabolism 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 and models are evaluated for their appropriateness. For each critical effect identified for a particular agent, separate DDEF analyses are conducted for UF_{AK} and UF_{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

The choice of the dose metric is an important component of the UF_{TK} . This choice depends on whether toxicity is best ascribed to a momentary or transient tissue exposure or a

1 cumulative dose to target tissue. For a given chemical, the appropriate dose metric will also be
2 determined by, and can vary with, both the duration of exposure and the adverse effect of
3 concern (U.S. EPA, 2006b). Selection of an appropriate dose metric based upon specific
4 endpoints involves several elements including these which are described in more detail below.

- 5
- 6 • Duration of exposure and effect;
- 7 • Identification of the active chemical moiety;
- 8 • Selection of the organ or tissue group in which some measure of internal dose is desired;
9 and
- 10 • Selection of the measure of exposure that best correlates with toxicity.

11
12 Whether an adverse effect is a consequence of acute or chronic exposure impacts the
13 choice of dose metric. For acute, reversible effects (e.g., sensory irritation, narcosis), a measure
14 of instantaneous or peak tissue exposure such as C_{\max} may be the most appropriate dose metric
15 (Alarie, 1973; Boyes et al., 2005). For chronic effects, in the absence of MOA information to the
16 contrary, it is generally assumed that some integrated cumulative measure of tissue exposure to
17 active toxicant is the most appropriate dose metric, e.g., area under the curve (AUC). Alternative
18 choices such as amount of chemical or rate of metabolite production can be used as appropriate
19 for a particular agent or MOA (U.S. EPA, 2006b). For example, there may be a case where a
20 temporally large influx of active chemical to a target site in a relatively short period of time
21 (peak exposure) is observed, in which case a less commonly used metric such as time above a
22 critical concentration (TACC) may be most appropriate. Data and rationale in support of a
23 particular dose metric need to be presented.

24
25 Clearance, while not typically considered a dose metric, can be useful in DDEF
26 derivation. Clearance is mathematically inversely related to AUC (e.g., $AUC = \text{dose}/\text{clearance}$),
27 thus differences in clearance values can be used in calculation of ratios. When metabolism
28 represents the primary or sole clearance mechanism, either of two clearance models may be
29 applicable. Intrinsic clearance (Cl_{int}) has been used for interspecies scaling of administered
30 doses in drug development (Houston and Carlile, 1997). Cl_{int} is calculated as V_{\max}/K_m , and is in
31 units of volume cleared of the substrate per unit time. Cl_{int} can be extrapolated to the whole body
32 with knowledge of protein binding and the recovery of the protein, cellular or subcellular
33 fraction used in the *in vitro* investigations (Carlile et al., 1997). Hepatic clearance (Cl_{hep}) is also
34 based on V_{\max}/K_m measurements, but also includes a substrate delivery term, whose value is
35 governed by hepatic blood flow. These measures of clearance differ in that Cl_{int} is not bounded
36 by hepatic blood flow, but Cl_{hep} cannot exceed hepatic blood flow. While metabolic rate
37 constants (V_{\max} and K_m) derived from *in vitro* data can also be scaled up and incorporated into
38 PBPK models, the use of these clearance models is a simpler approach useful when an
39 appropriate PBPK model is unavailable. Classical, compartmental TK analyses and measures of
40 clearance are best suited for conditions where metabolism represents a detoxication process,
41 when substrate concentration is less than the K_m value and when metabolism represents the
42 major clearance mechanism.

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

10
11 The organ or tissue group where the toxic effects occur is ideally the site from which
12 estimates of internal dose (tissue concentration) are generated. In practice this information may
13 be unavailable in the absence of an appropriate PBPK model. It may be necessary to use
14 absorbed dose of the parent chemical as a surrogate measure of internal dose. Another surrogate
15 dose metric is measurement of parent chemical or active metabolite in circulating blood if the
16 relationship between target tissue dose and blood is known or can be reliably inferred from
17 experimental data. Some data have demonstrated that blood:air partition coefficient values may
18 vary appreciably between species, but that tissue:air (e.g., liver:air) partition coefficients are
19 similar between mammalian species (Thomas, 1975). It seems reasonable to use the cross-
20 species similarity in the primary determinant of diffusion from blood into tissues as a
21 justification to rely on concentrations of the toxicant in blood as a surrogate for tissue
22 concentrations. However, when local tissue bioactivation may be a determinant of the toxic
23 response, this should be given careful consideration. Those issues notwithstanding, measures of
24 internal dose in circulating blood (see IPCS, 2005) may be used as the basis for DDEF derivation
25 under either of these conditions:

- 26
27 • When evaluating interspecies differences, the distribution from blood to sensitive
28 (critical) tissues is shown to be or can be assumed to be the same between animals and
29 humans
- 30 • When evaluating intraspecies differences, the distribution from blood to sensitive
31 (critical) tissues is shown to be or can be assumed to be the same between members of
32 the general human and potentially sensitive human groups
33

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

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

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 UF_{AK} or UF_{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 UF_{AK} or UF_{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, it is important when deriving UF_{AK} and UF_{HK} to consider interspecies differences in ADME. *In vitro* data should be used for quantitative purposes only when interpreted in the context of the intact system, as discussed in Section 2.2.3.2. Care must be taken to avoid taking isolated findings out of context.

3.2. INTERSPECIES TOXICOKINETIC EXTRAPOLATION (UF_{AK})

This section provides a discussion of the quantitative differences in the TK between animals and humans that are used for interspecies extrapolation (UF_{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 (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.

3.2.1. Considerations for UF_{AK}

Extrapolation of TK data obtained in animal models to humans (UF_{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).

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

Toxicokinetics

INTERSPECIES EXTRAPOLATION

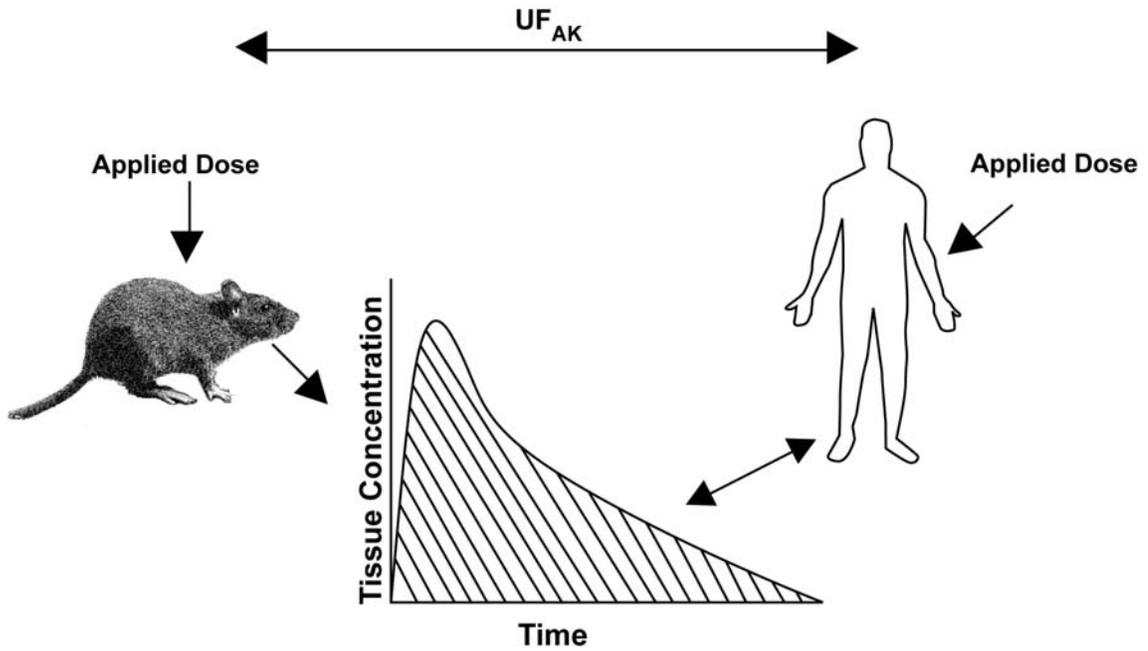


FIGURE 3

1
2
3
4
5
6 Interspecies Toxicokinetics. In keeping with guidance set for inhaled substances (U.S. EPA,
7 1994), interspecies differences in toxicokinetics are defined as differences in the external dose
8 producing the same level of the dose metric in the target tissue of interest in test animals and in
9 humans representative of the general population. Because humans are considered more sensitive
10 than animals, UF_{AK} is the ratio of animal dose:human dose. For clarity in presentation, UF_{AK} is
11 applied to the external dose in animals.

1 be careful attention paid both to measures of central tendency and to variability, particularly in
 2 the range of concentrations or doses close to the point of inflection (where the shape or slope of
 3 the dose response curve changes) because of potential non-linearities in metabolism.

4 5 **3.2.2. Computation**

6
7 For TK extrapolation, the goal is to determine differences in dosimetry between groups.
 8 For interspecies extrapolation, toxicokinetically equivalent exposures (doses) are determined by
 9 fixing the internal dose (level of the dose metric at or near the POD) and determining the ratio of
 10 external (applied doses) that result in the same level of the dose metric in animals and humans.
 11 This approach is consistent with that in the RfC guidance for inhalation toxicants (U.S. EPA,
 12 1994).

13 14 **3.2.2.1. Use of TK Models**

15
16 A PBPK or other TK model provides the most biologically appropriate approach for
 17 evaluating interspecies TK extrapolation. The model is subject to evaluation as described in
 18 Section 2.2.2. The model can be used in different ways, depending on the model and the
 19 circumstances. In some cases, the TK model may be used directly to perform interspecies
 20 extrapolation, derive a Human Equivalent Concentration or Dose that includes TK
 21 considerations, thus alleviating the need for UF_{AK} . In other cases, the TK model may be used to
 22 derive UF_{AK} .

23 24 **3.2.2.2. Use of Ratios**

25
26 When AUC or concentration at steady state is the relevant dose metric, and if advanced
 27 TK models are not available, UF_{AK} can be derived using a ratio of doses producing the same
 28 AUC value. This is accomplished by identifying external doses associated with the AUC value
 29 produced in animals at the point of departure (AUC_A), demonstrated below (Equation 1 and
 30 Figure 4). The human dose that produces the same AUC value as observed in animals is the TK
 31 equivalent dose. In these cases, differences between the animal and the human dose producing
 32 the same AUC value in each species are captured as the DDEF. See below:

$$33 \quad \text{AUC} = \text{Dose}/\text{Clearance} \quad (\text{Eq. 1})$$

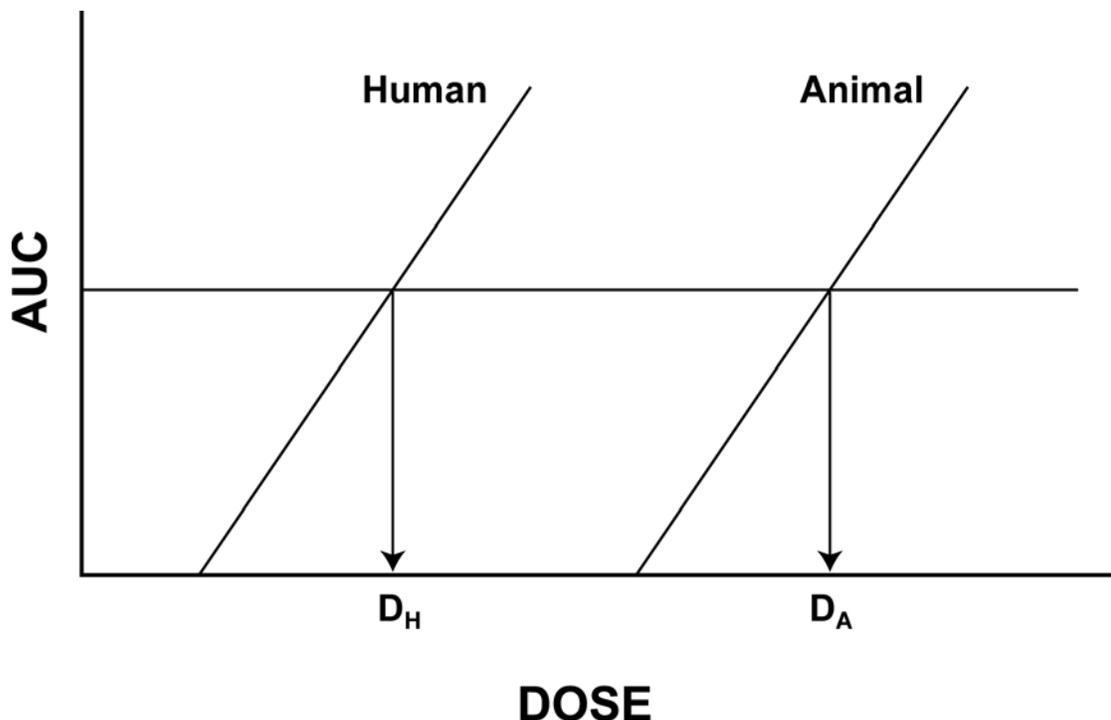
$$34 \quad \text{AUC}_A = D_A/Cl_A = D_H / Cl_H$$

$$35 \quad \text{UF}_{AK} = \frac{D_A \text{ or } Cl_A}{D_H \text{ or } Cl_H}$$

36
37
38
39
40 Using AUC as an example, the value for the dose metric would be AUC_A, that is the
 41 AUC value determined in animals at the POD. Thus in this example, Equation 1 becomes

$$42 \quad \text{UF}_{AK} = D_A \text{ producing AUC}_A / D_H \text{ producing AUC}_A$$

1



2

3

4

5

6

7

FIGURE 4

8 Interspecies Extrapolation Based on AUC. In this example, AUC is the appropriate dose metric,
 9 and the relationship between dose and AUC is determined in animals and in humans. This
 10 method can be used to develop a Human Equivalent Dose or Concentration, or in the calculation
 11 of UF_{AK} . Calculation of UF_{AK} requires knowledge of applied doses in animals (D_A) and in
 12 humans (D_H) that produce the AUC value determined in animals at the point of departure
 13 (AUC_A).

1 where

2 D_H = the human external dose

3 D_A = the animal external dose

4 UF_{AK} = uncertainty factor for interspecies extrapolation covering toxicokinetics

5 $Dose_A$ = administered or external dose to the animal

6 $Dose_H$ = administered or external dose to the human

7 AUC_A = area under the curve in the animal

8

9 This is graphically presented in Figure 4.

10

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

16

17 3.2.3. Relationship to Other U.S. EPA Guidance

18

19 The development and use of advanced information on tissue dosimetry to serve as the
20 basis for quantitative, non-default uncertainty factors is consistent with existing U.S. EPA
21 guidance and philosophy (U.S. EPA, 2002, 2006b). The U.S. EPA's Inhalation Reference
22 Concentration Methodology espouses a continuum of approaches from complete lack of
23 knowledge to biologically based dose-response models (U.S. EPA, 1994). Under this approach,
24 there are two processes that each cover the interval between lack of knowledge and knowledge
25 of target tissue and target tissue concentrations in test species and humans. The first is for
26 reactive (Category 1 gases) gases and inhaled particles that damage portal of entry (respiratory
27 tract) tissues, and the second is for gases that are absorbed and produce their toxicity in tissues
28 bathed by circulating blood (Category 3 gases). Under the first approach, toxicity information
29 identifies the affected region of the respiratory tract and species differences in regional
30 respiratory tract surface area and air flow (respiratory rate) serve as the basis to quantify species
31 differences in dosimetry. In the second approach, species differences in the solubility of the
32 compound in blood (the blood:air partition coefficient) between species serves as the basis upon
33 which to quantify species differences in dosimetry. More recent guidance (U.S. EPA, 2006a) on
34 orally encountered toxicants instructs the application of dose scaling by the ratio of species body
35 weights raised to the $3/4$ power (human BW:animal BW)^{3/4}. Each of these guidance documents
36 indicates that their approaches are intermediates, to be superseded when more detailed
37 information on tissue dosimetry can be developed. The subject of the present guidance is the
38 development and interpretation of quantitative toxicokinetic data for the purpose of developing
39 non-default values for inter- and intraspecies uncertainty/extrapolation.

40

41 3.2.4. Conclusions for UF_{AK}

42

43 Mathematically, the DDEF may be the ratio of the external doses in animals at or near the
44 POD to the value at a central tendency measure of the general human population that each result
45 in the same level of the dose metric (this represents developing the Human Equivalent
46 Concentration or the Human Equivalent Dose). UF_{AK} values may be calculated for multiple

1 organs/effects. The same PODs should be used for test animals and humans for a given
2 organ/effect. Quantitatively, DDEF values for UF_A components might be less than 1 if humans
3 are less sensitive.

4
5 The risk assessor provides a summary of all conclusions and their scientific support. This
6 includes description of toxicity data identifying the target tissue, chemical species, MOA and
7 species concordance of effects. Data describing the TK, metabolism of the compound and
8 relationship between administered dose and internal dose are also summarized. Data that show a
9 linear relationship between external dose and internal dose metrics can be specifically reiterated
10 in this description; this will indicate generalizability of the UF_{AK} to doses that may be higher or
11 lower than those used in its calculation. A comparison of results from multiple target organs will
12 increase confidence in the developed DDEF value for the critical organ. Results can be
13 presented in tabular form for ease of comparison across endpoints.

14 15 **3.3. INTRASPECIES TOXICOKINETIC EXTRAPOLATION (UF_{HK})**

16
17 This section provides a discussion of the quantitative differences in the TK among
18 humans for intraspecies extrapolation (UF_{HK}). From a toxicokinetic standpoint, among humans
19 experiencing the same external dose, susceptibility is due to higher target tissue concentrations of
20 the toxicant in some individuals or groups relative to the majority of the human population. TK
21 differences for intraspecies extrapolation are characterized as the ratio of an internal dose metric
22 attained in the general human population to the dose metric from susceptible humans exposed to
23 the same external dose or concentration.

24 **3.3.1. Considerations for UF_{HK}**

25 26 **3.3.1.1. *Susceptible Groups or Individuals***

27
28 Potentially susceptible groups/lifestages (e.g., children, elderly or age-related
29 susceptibility or those with a disease making them susceptible to a toxicant) can be identified. In
30 some instances, individuals may have a condition or difference in some physiologic or
31 biochemical process that may be a deterministic factor in dosimetry that serves as the basis for
32 assumed sensitivity (e.g., polymorphism of a given metabolizing enzyme). In these cases, the
33 distribution of the dose metric among the population will not have a unimodal distribution (see
34 Figure 5, left panel). In other cases, sensitivity may be distributed throughout the general
35 population, and sensitive individuals may be those in the tail of the distribution (i.e., unimodal;
36 see Figure 5, right panel). Documenting this information and/or these assumptions serves as the
37 basis for selecting a unimodal or a bimodal distribution of sensitivity.

38 39 **3.3.1.2. *Target Tissues***

40
41 When responses are observed in several organs at or near the same dose level, a
42 comparison of UF_{HK} values developed for those tissues will be informative. However, the
43 selection of a target organ for calculating human variability other than the one serving as the
44 basis for animal to human extrapolation, needs to be accompanied with a justification. This also
45 extends to instances in which human studies identify effects in an organ or tissue and variability

INTRASPECIES
EXTRAPOLATION

Toxicokinetics

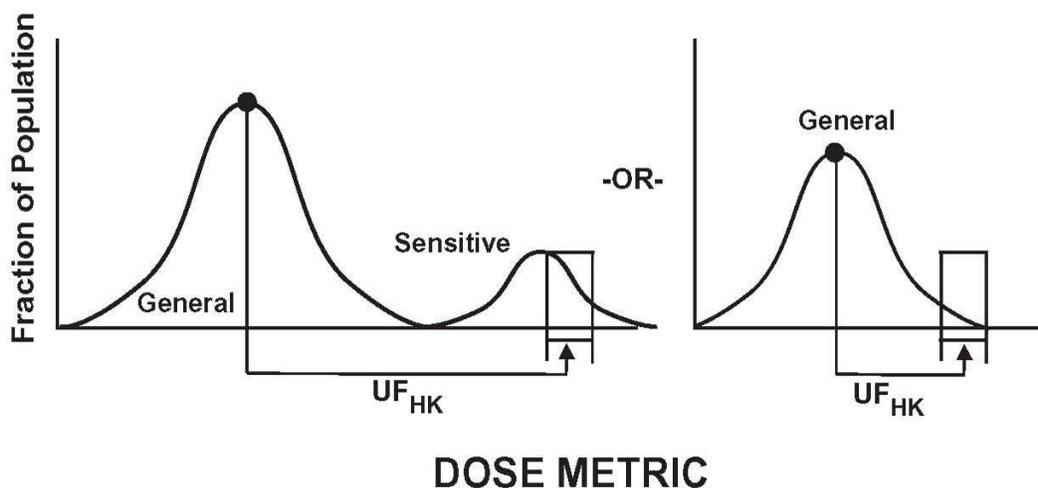


FIGURE 5

1
 2
 3
 4 Intraspecies Toxicokinetics. From a toxicokinetic standpoint, susceptibility is based on attaining
 5 higher target tissue concentrations of the toxicant. For this evaluation, a fixed exposure is
 6 studied and human interindividual variability is measured as differences in the dose metric.
 7 When a group of the population can be identified as potentially susceptible, toxicokinetic data
 8 from that group can be compared to the general population (left panel). UF_{HK} should be
 9 determined as the ratio of the level of the dose metric at some point in the upper 50% of the
 10 distribution (i.e., 95, 97.5, 99th percentile) for those deemed sensitive to the level of the dose
 11 metric at a central tendency measure of the general population. However, when potentially
 12 susceptible individuals may represent a small percentage of the general population (when no
 13 specifically identifiable potentially sensitive groups can be identified, or when toxicokinetic
 14 studies disprove the hypothesis of toxicokinetic sensitivity of the identified group), then a
 15 slightly different analysis is conducted (right panel). In this case, UF_{HK} should be determined as
 16 the ratio of the level of the dose metric at a percentile of the population considered sensitive (i.e.,
 17 95, 97.5, 99th percentile) to the level of the dose metric at a central tendency measure of the
 18 general population.

1 is assessed in another organ or tissue. The extrapolation approach will be perceived as most
2 consistent when the same tissue or organ is used for each phase of the extrapolation procedure.

3 4 **3.3.1.3. Dose Response**

5
6 Because variability in internal dosimetry may be a function of dose, the selection of the
7 external exposure (e.g., inhaled concentration or orally ingested dose) is important.
8 Conceptually the animal POD is first extrapolated to produce a toxicokinetically equivalent
9 human dose or concentration. The DDEF for interspecies differences in TD should be applied to
10 the tissue concentration defining the Human Equivalent Exposure to complete interspecies
11 adjustment. It is this species-adjusted concentration that is most appropriate for application in
12 the evaluation of human interindividual variability.

13
14 The basis for comparison of human variability is at the level of the internal, rather than
15 the external dose. Thus, it is important that the relationship between internal and external doses
16 be well characterized. Comparisons of the external doses that produce the same level of the
17 internal dose (between susceptible and non-susceptible groups) can be substantially complicated
18 when there are non-linearities in the relationship between external dose and internal
19 concentration. This is particularly difficult when non-linearity of dose is for one group and not
20 for the other. This complication can be avoided when the comparison is made as difference in
21 internal doses developed from the same external exposure.

22
23 Specific differences among humans, particularly those demonstrated *in vitro* (i.e.,
24 intrinsic clearance), are most reliable when they are translated into differences in dosimetry—
25 through the development of different levels of the dose metric of interest.

26 27 **3.3.2. Computation**

28
29 For intraspecies TK extrapolation, differences in dosimetry are characterized for human
30 groups or lifestyles by comparison to the general population. Thus the comparisons are among
31 differences in internal dosimetry (or target site dose) resulting from the same external exposure.
32 To address human variability, some attention must be devoted to identifying a susceptible
33 population group or a fraction of the general population considered to be sensitive. TK data are
34 then analyzed to determine values for the dose metric in the general and sensitive groups.

35 36 **3.3.2.1. Use of TK Models**

37
38 A PBPK or other TK model provides the most robust approach for evaluating
39 intraspecies TK extrapolation. The model is subject to evaluation as described in Section 2.2.2.
40 When an appropriate model is available, the model can be used in different ways depending on
41 the model. In some cases, the TK model may directly account for within human variation and/or
42 include data from the sensitive group thus elevating the need for UF_{HK} . In other cases, the TD
43 may be used to derive UF_{HK} .

1 **3.3.2.2. Use of Ratios**

2
 3 Where TK models are not available, UF_{HK} can be derived using a ratio (Equation 2). The
 4 value for the dose metric employed for the general population (e.g., AUC_{gen}) would be that level
 5 of the dose metric identified from UF_{AK} studies, divided by the DDEF value for UF_{AD}. The
 6 value for the dose metric in sensitive individuals (e.g., AUC_{%ile}) should be determined from
 7 empirical data or pharmacokinetic modeling.
 8

9
$$UF_{HK} = \frac{AUC_{\%tile} \text{ or } Cl_{gen}}{AUC_{gen} \text{ or } Cl_{\%tile}} \quad (\text{Eq. 2})$$

10 where

- 11 UF_{HK} = uncertainty factor for intraspecies extrapolation covering toxicokinetics
- 12 AUC_{gen} = area under the curve at a measure of central tendency in the general human
 13 population
- 14 AUC_{%tile} = area under the curve at a percentile of interest in the sensitive human population
- 15 Cl_{gen} = clearance at a measure of central tendency in the general human population
- 16 Cl_{%tile} = clearance at a percentile of interest in the sensitive human population

17
 18 Because clearance values are the mathematical reciprocal of internal dose (AUC), they
 19 may also be used to calculate a DDEF value. Because susceptible humans may be assumed to be
 20 more sensitive than general humans (e.g., they clear toxicants more slowly), the sensitive human
 21 clearance value remains in the denominator.
 22

23 **3.3.3. Conclusions for UF_{HK}**

24
 25 The UF_{HK} is essentially a comparison of internal doses resulting from the same external
 26 exposure to both the general population and sensitive individuals. When using an empirical
 27 ratio, the UF_{HK} is the ratio of the dose metric value at a percentile of the distribution intended to
 28 represent sensitive individuals to the dose metric value at a central tendency measure of the
 29 general population. Quantitatively, DDEF values for UF_H components cannot be less than 1.
 30

31 The dose selected for quantifying human interindividual variance may have an impact on
 32 the magnitude of variability. The dose adjusted from the animal POD is the preferred dose for
 33 quantitation of human variability. Increased confidence is developed when the relationship of
 34 the human dose is compared with that developed from a complete application of interspecies
 35 extrapolation factors. While DDEF values may be calculated for multiple organs/effects (or even
 36 doses), the same external dose needs to be used for the sensitive and the general populations.
 37

38 The risk assessment describes the mathematical method to be employed, the type of
 39 distribution and percentile(s) of interest; identifies the dose metrics (e.g., AUC of parent
 40 compound in kidney), and the target tissues for which UF_{HK} are developed. Confidence in the
 41 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. This section provides a discussion of factors common to interspecies and intraspecies extrapolation for TD. In contrast to TK, which focuses on *differences in internal dosimetry*, TD differences are quantified as *differences in concentration⁶ 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.

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

- 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 which describe population variation? Do these data identify a sensitive group(s) or lifestages? Can the degree of this sensitivity be estimated?

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.

1 4.1.1. Mode of Action

2
3 TD extrapolation should be endpoint-driven (considered in the context of the toxic
4 endpoints most relevant for purposes of risk assessment). The choice of critical effect should be
5 justified, when possible, based on findings of response in exposed humans. Understanding MOA
6 for the agent(s) of interest helps to ensure that the TD responses used to derive the DDEF will be
7 robust scientifically. These responses could include receptor affinity, enzyme inhibition and
8 molecular changes, among others. Repair of DNA or tissue damage, biological thresholds,
9 residual function and other processes that could contribute to non-linearity in effect are
10 considered and discussed. Experimental systems and measured responses should be the same or
11 comparable.

12 13 4.1.2. Relating Response to Dose or Concentration

14
15 TD data may be developed from the results of *in vivo* or *in vitro* studies. In some cases,
16 *in vivo* data may be used, but care should be taken to control for the influence of toxicokinetics.
17 If there are existing human data measuring the response, then these data can be used to derive the
18 POD, thereby removing the need for the UF_A . Likewise, if there are data from the most sensitive
19 human group, those data could be used for the risk assessment, and an UF_H may not be needed.

20
21 Care should be taken to assure that kinetic factors do not confound the interpretation of
22 response data. Comparisons based on internal dose metric are preferred over applied dose; that
23 is, concentration producing the level of response (rather than applied dose or concentration) is
24 preferred so that TD response may be distinguished from TK differences. Tissue-specific
25 metabolism could also influence the actual target concentrations and must be considered. Blood
26 levels are an acceptable measure of internal dose when it can be shown that they are proportional
27 to concentrations of toxicant in target tissue or biological preparation.

28
29 When using *in vitro* systems, the response measured should be representative of the
30 toxicity; that is, the measured endpoint should be the same as or highly related to the critical
31 effect or key event. In cases where the measured response differs, it is important to describe the
32 potential impact of these differences on the final DDEF. When using *in vitro* data, the
33 comparability of chemical uptake between animal and human tissues should be demonstrated. *In*
34 *vitro* results should include data describing the test chemical metabolism by the system used to
35 generate response data.

36 37 4.1.3. Range of Doses or Concentrations

38
39 The relationship between the doses, tissue concentrations and/or *in vitro* concentrations
40 used to derive the DDEF, and those attained in the toxicity studies characterizing the POD,
41 should be characterized. Optimally, the concentrations used in studies characterizing response
42 include the concentration at the POD. This is important because the variability in the response
43 may change with increasing or decreasing dose or concentration. Doses and/or tissue
44 concentrations may also be compared to those expected from environmental exposure; this is
45 part of the consideration of overall relevance of the test system.

4.2. INTERSPECIES TOXICODYNAMIC EXTRAPOLATION (UF_{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 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 (Figure 6).

4.2.1. Considerations for UF_{AD}

4.2.1.1. Mode of Action

Endpoint(s) must be chosen from which to derive the UF_{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

Confidence is increased when the response level employed for UF_{AD} calculation approximates the response level at the POD. 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. When the dose response curves are parallel, then at many response levels the magnitude of the UF_{AD} will not vary significantly between low and high doses. However, if the shapes of the curves are different, then the magnitude of UF_{AD} will depend on the response

1 level selected. Note that concentrations causing greater magnitudes of effect may also produce
2 non-specific cellular changes that could confound the comparisons.

3 4 **4.2.1.4. *In Vitro* Data**

5
6 When using data from *in vitro* systems, the activity of the parent chemical and/or
7 metabolites, as well as the extent of metabolism of the compound by the *in vitro* system, should
8 be known. The *in vitro* assay should measure a response that can be linked to the toxic outcome,
9 and the assays should employ the same (responding) tissues from the *in vivo* test animal
10 species/strain/sex/lifestage and from humans. Experimental systems should be as closely
11 matched between species as possible, and the concentrations of toxicant *in vitro* should be
12 compared to tissue concentrations (the dose metric) at the POD. See Section 2.2.3.2 for other
13 general considerations.

14 15 **4.2.2. Computation**

16
17 For TD extrapolation, the goal is to determine the difference between test groups on the
18 basis of dose metric value or concentration producing the same response level. For interspecies
19 TD extrapolation (UF_{AD}), the *in vitro* or *in vivo* target tissue concentration corresponding to
20 response level is determined for both the test species and humans representing the responding
21 population (typical of the species/strain/sex/lifestage). Preferably, data are sufficient for dose-
22 response modeling (e.g., benchmark dose [BMD] modeling) when doses or concentrations do not
23 produce response levels near that intended for extrapolation. The data should support derivation
24 of the same specified levels of response (e.g., ED_{10} or BMD_{10}) in the species or systems being
25 compared. If the data are not amenable to response modeling, it is recommended that the
26 response level selected for DDEF derivation be the same (or close to the same) in both test
27 species and humans.

28 29 **4.2.2.1. *Use of TD Models***

30
31 A biologically based dose-response or other TD model provides the most robust approach
32 for evaluating interspecies TD extrapolation. The model is subject to evaluation as described in
33 Section 2.2.2. When available, the model can be used to inform several decisions, depending on
34 the model. In some cases, the TD model may be used directly to perform interspecies
35 extrapolation and to derive a Human Equivalent Concentration or Dose that includes TD
36 considerations, thus alleviating the need for UF_{AD} . In other cases, the TD may be used to derive
37 UF_{AD} is used.

38 39 **4.2.2.2. *Use of Ratios***

40
41 When PD models are not available, UF_{AD} would be calculated as a ratio describing the
42 relationship between the tissue concentrations producing a set response in human compared to
43 animal tissues, preferably at, or near, the response level at the POD. For example, “x response
44 level” may be a 10% response if ED_{10} or BMD_{10} is used. The UF_{AD} would be calculated as
45 follows:

INTERSPECIES
EXTRAPOLATION

Toxicodynamics

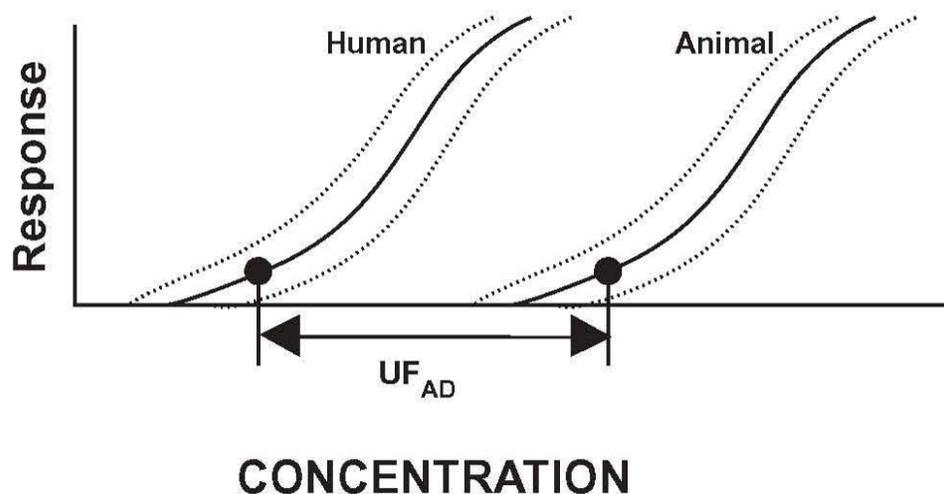


FIGURE 6

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.

$$UF_{AD} = \frac{\text{Concentration}_{A-x \text{ response level}}}{\text{Concentration}_{H-x \text{ response level}}} \quad (\text{Eq. 3})$$

where

UF_{AD} = uncertainty factor for interspecies extrapolation covering toxicodynamics

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

$\text{Concentration}_{H-x \text{ response level}}$ = 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 UF_{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 UF_{AD}

Mathematically, the UF_{AD} will be the relationship of the concentrations or dose metric values resulting in the same level of response in both the test species and the generally-responding human. 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 UF_{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, UF_{AD} can be less than 1 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 UF_{AD} value can be generalized to doses that may be higher or lower than those used in its calculation.

4.3. INTRASPECIES TOXICODYNAMIC EXTRAPOLATION (UF_{HD})

This section describes derivation of the UF_{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.

1 4.3.1. Considerations for UF_{HD}

2 3 4.3.1.1. Susceptible Groups or Individuals

4
5 Susceptibility in the human population may be due to life stage, health status or disease
6 state, genetic disposition or other factors. Considering susceptibility to more than one critical
7 effect may require consideration of more than one life-stage; critical windows of development,
8 and, therefore, windows of susceptibility, occur at different times for various tissues, organs and
9 systems. Currently, sufficient data to address susceptibility are rarely available; however,
10 research in this area is rapidly expanding. For example, population variation, such as genetic
11 polymorphisms, is an expanding area of study. It is anticipated that the increased availability
12 and experience applying “omics” technologies will benefit the derivation of DDEFs in general
13 and UF_{HD} in particular. A data-derived UF_{HD} is feasible given human data are of sufficient
14 quality; the data address aspects of the critical effect consistent with that identified from
15 applicable human or animal studies; and the studies have been conducted in the segment(s) of
16 individuals or the population deemed sensitive.

17
18 Ideally, data will be robust enough to enable more than point estimates in the general and
19 susceptible groups. As discussed in more detail below, distributional analysis of response data
20 should be conducted to identify points for use in quantitation. The relationship between the
21 measured response and the toxicity endpoint of concern (e.g., critical effect or key event) should
22 be described, whether determined *in vivo* or *in vitro*.

23 24 4.3.1.2. Target Tissues

25
26 For calculation of UF_{HD}, data for multiple responding tissues can be evaluated and
27 multiple DDEFs derived. It is particularly important to evaluate those tissues that demonstrate
28 response at doses or concentrations near those for the critical effect.

29 30 4.3.1.3. In Vitro Data

31
32 Given the constraints on generation of human response data *in vivo*, *in vitro* studies offer
33 an appealing alternative. Samples selected for *in vitro* investigation should represent the general
34 human population as well as and those groups or individuals thought or demonstrated to be
35 susceptible. See Section 2.2.3.2 for other general considerations.

36 37 4.3.2. Computation

38
39 For TD extrapolation, the goal is to determine the difference between humans on the
40 basis of concentration producing the same response level. For quantitation, data on the critical
41 response(s) are derived from a population that includes susceptible groups or individuals.
42 Because the data available to define potentially susceptible groups or individuals could be
43 viewed in different ways, a statistical analysis may be helpful to determine distribution type (see
44 Figure 7):

Toxicodynamics

INTRASPECIES
EXTRAPOLATION

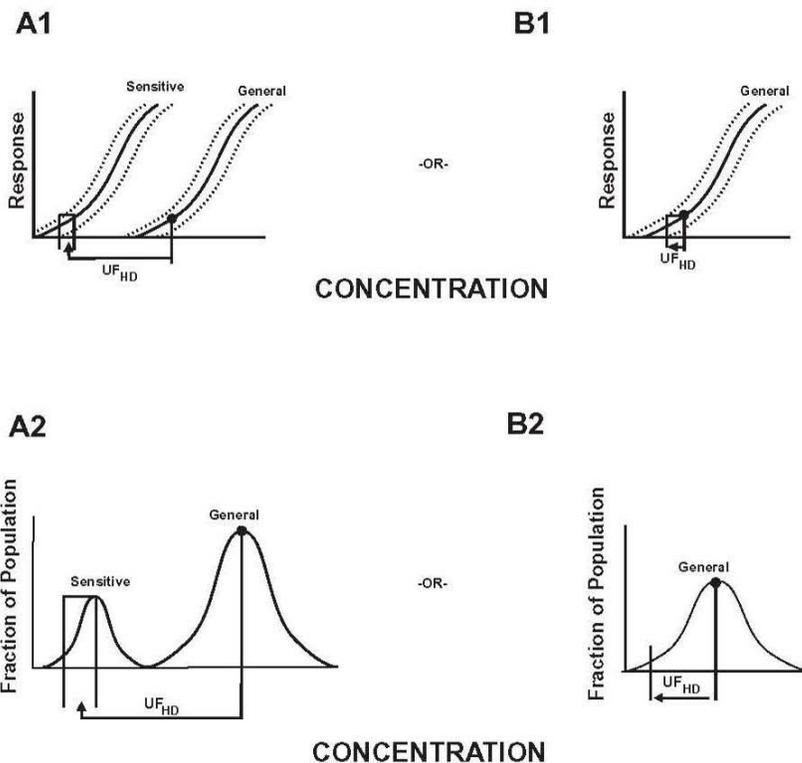


FIGURE 7

1
 2
 3
 4 Intraspecies Toxicodynamics. DDEF values for TD are defined by ratios of concentrations producing the
 5 *same level of response* in the general population and some defined percentile (e.g., 1st, 2.5th, 5th, etc.) for
 6 the distribution representing sensitive individuals. When a specific group of the population can be
 7 identified as potentially susceptible, TD data from that group can be compared to the general population
 8 (panels A1 and A2). Panel A1 presents a dose-response curve (cumulative distribution plot) for both
 9 populations that demonstrates the central tendency (solid line) and confidence bounds, or bounds of
 10 variability, (dashed lines) for data obtained from the general population and from an identifiable sensitive
 11 group. In this example, the level of response (Y-axis) has been selected (e.g., 10% response level), and
 12 the concentrations producing this level of response in the general and sensitive populations/groups are
 13 obtained from the X-axis. Panel A2 is derived from the same data used for panel A1, but it presents the
 14 distribution of concentrations producing the defined level of response only; no other dose-response data
 15 are carried over into panel A2. Alternately, when potentially susceptible individuals represent a small
 16 percentage of the general population (panels B1 and B2), a slightly different analysis is conducted. In this
 17 case, UF_{HD} should be determined as the ratio of the concentrations producing the same level of response
 18 (1) at a measure of the central tendency in the population to (2) the concentration producing the response
 19 level at a percentile of the general population considered sensitive (e.g., 1, 2.5, 5th percentile). Panel B1
 20 demonstrates this comparison using the concept of confidence bounds on the dose-response relationship
 21 and panel B2 demonstrates the distribution of concentrations producing the response, only at the response
 22 level chosen for comparison (e.g., the 10% response level).

- 1 • A unimodal distribution where the potentially susceptible group(s) represent the tail of
- 2 the distribution because they can not be separated from the general population
- 3 • A bimodal (or multimodal) distribution where the group(s) can be readily identified
- 4

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

7 4.3.2.1. *Use of TD Models*

10 A biologically based dose-response or other TD model provides the most robust approach
11 for evaluating intraspecies TD extrapolation. When sufficient data are available, these TD
12 models can be structured and exercised to include differences in mode of action components that
13 may be lifestage-dependent or influenced by other potentially susceptibility-inducing conditions
14 such as genetic polymorphisms. The model is subject to evaluation as described in Section 2.2.2.
15 Specific to UF_{HD}, it is critical that the model parameter reflecting the underlying cause of
16 susceptibility in a group be well documented. When an appropriate model is available, the
17 model can be used in different ways depending on the model. In some cases, the TD model may
18 directly account for within human variation and/or include data from the sensitive group thus
19 elevating the need for UF_{HD}. In other cases, the TD may be used to derive UF_{HD}.

21 4.3.2.2. *Use of Ratios*

23 When TD models are not available and there are groups or individuals that can be
24 identified as sensitive, then the UF_{HD} may be defined as the ratio between the concentrations
25 producing the same level of response in the general population and a lower percentile in the
26 sensitive group (see Figure 7) using Equation 4:

$$28 \quad UF_{HD} = \frac{\text{Concentration}_{gen}}{\text{Concentration}_{sens \%tile}} \quad (\text{Eq. 4})$$

30 where

31 UF_{HD} = uncertainty factor for intraspecies extrapolation covering
32 toxicodynamics

33 $\text{Concentration}_{gen}$ = concentration producing the in the general human population

34 $\text{Concentration}_{sens \%tile}$ = concentration producing the response at a percentile of interest for
35 the sensitive group

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

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

10 11 **4.3.3. Conclusions for UF_{HD}**

12
13 A biologically based dose-response or other TD model provides the most robust approach
14 for evaluating intraspecies TD extrapolation. When using empirical ratios, the UF_{HD} will be the
15 ratio of the concentration producing the specified level of response in the general human
16 population to the concentration producing the same level of response in susceptible groups or
17 individuals. Increased confidence in the UF_{HD} is developed when the concentration used for the
18 comparison of responses is compared to doses or concentrations at the POD. Quantitatively,
19 UF_{HD} cannot be less than 1.

20
21 The risk assessor describes all choices and their rationales, including the use of multiple
22 response levels, critical effects (or key events), analytical methods or data from susceptible
23 groups or individuals. The conclusions include a clearly worded description of the mathematical
24 method(s) employed and a presentation of the relationship between the measured response and
25 toxicity (i.e., critical effects or key events). This description should clearly identify and provide
26 the justification for available data and points in the distribution(s) representing sensitive
27 individuals. Attention should be paid to characterizing the distribution type employed for
28 analysis; uncertainty in the choice of distribution type can be allayed by presenting DDEF values
29 resulting from multiple distribution types.

5. FINAL STEPS

The composite DDEF is calculated after the risk assessor has derived the appropriate DDEFs for inter- and/or intraspecies differences in TK and TD. The composite factor is calculated by multiplying the specific UFs (default and/or DDEFs), as shown in Equation 5. 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.

$$CF = UF_{AK} \times UF_{AD} \times UF_{HK} \times UF_{HD} \quad (\text{Eq. 5})$$

where

- CF = composite uncertainty factor
- UF_{AK} = uncertainty factor for interspecies extrapolation covering toxicokinetics
- UF_{AD} = uncertainty factor for interspecies extrapolation covering toxicodynamics
- UF_{HK} = uncertainty factor for intraspecies extrapolation covering toxicokinetics
- UF_{HD} = uncertainty 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 UF_{AK} but not UF_{AD}). In these cases the remaining extrapolation is done by an appropriate default procedure. As such, DDEFs and defaults are used in combination. Often this default will be a 3× UF—as described in the existing RfC methodology and the $\frac{3}{4}$ body weight procedure (U.S. EPA, 1994, 2006a). When data are not available to develop DDEFs for either component of interspecies or intraspecies extrapolation, the 10× default factor is applied.

Finally, the composite factor provides the total magnitude of UFs. The values derived for each 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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