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

Office of the Science Advisor
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Office of the Science Advisor
Risk Assessment Forum
U.S. Environmental Protection Agency
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LIST OF ABBREVIATIONS

ADME	absorption, distribution, metabolism, excretion
Agency	U.S. Environmental Protection Agency
AOP	adverse outcome pathway
AUC	area under the curve (optional subscripts $_{A}$ = animal AUC value; $_{gen}$ = general human population AUC value; $_{sens}$ = sensitive human population AUC value)
AUC_A	animal AUC value (AUC value produced in animals at or near the animal point of departure)
AUC_{gen}	general human population AUC value (area under the concentration-time curve at a fixed external dose at a measure of central tendency in the entire or general human population)
AUC_{sens}	sensitive human population AUC value (area under the concentration-time curve at a fixed external dose at a percentile of interest for the sensitive human population or representing sensitive individuals among the entire human population)
BBDR	biologically based dose-response
$BMDL_x$	lower confidence bound on benchmark dose corresponding to an *x*% increase in response
$BW^{¾}$	body weight raised to the ¾ power
CF	composite factor
Cl	clearance (optional subscripts $_{A}$ = animal Cl value; $_{H}$ = human Cl value; $_{gen}$ = general human population Cl value; $_{sens}$ = sensitive human population Cl value; $_{hep}$ = hepatic Cl value; $_{int}$ = intrinsic Cl value)
Cl_A	animal Cl value
Cl_H	human Cl value
Cl_{gen}	general human population Cl value (at a measure of central tendency in the entire or general human population)
Cl_{sens}	sensitive human population Cl value (at a percentile of interest in the sensitive human population or representing sensitive individuals among the entire human population)
Cl_{hep}	hepatic Cl value
Cl_{int}	intrinsic Cl value
C_{max}	maximum concentration (optional subscripts $_{gen}$ = general human population C_{max} value; $_{sens}$ = sensitive human population C_{max} value)
$C_{max\ gen}$	general human population C_{max} value (at a fixed external dose at a measure of central tendency in the entire or general human population)
$C_{max\ sens}$	sensitive human population C_{max} value (at a fixed external dose at a percentile of interest in the sensitive human population or representing sensitive individuals among the entire human population)
CSAF	chemical-specific adjustment factor

LIST OF ABBREVIATIONS (continued)

D_A	animal external dose (administered or external dose in the test animal species that leads to a level of a toxicologically relevant dose metric at or near the point of departure)
D_H	human external dose (administered or external dose at the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species)
DDEF	data-derived extrapolation factor
DNA	deoxyribonucleic acid
EF	extrapolation factor (optional subscripts $_A$ = interspecies/animal to human; $_H$ = intraspecies/within human variability; $_D$ = toxicodynamic component; $_K$ = toxicokinetic component)
EF_{AD}	interspecies toxicodynamic EF
EF_{AK}	interspecies toxicokinetic EF
EF_{HD}	intraspecies toxicodynamic EF
EF_{HK}	intraspecies toxicokinetic EF
EPA	U.S. Environmental Protection Agency
HEC	human equivalent concentration
HED	human equivalent dose
IOM	Institute of Medicine
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
K_m	Michaelis constant, substrate concentration at rate of ½ V_{max}
MOA	mode of action
NRC	National Research Council
PBPK	physiologically based pharmacokinetic
POD	point of departure
RfC	reference concentration
RfD	reference dose
TD	toxicodynamic
TK	toxicokinetic
UF	uncertainty factor (optional subscripts $_A$ = interspecies/animal to human; $_H$ = intraspecies/within human variability)
UF_A	interspecies UF
UF_H	intraspecies UF
U.S. EPA	U.S. Environmental Protection Agency
V_{max}	theoretical maximal initial velocity
WHO	World Health Organization

AUTHORS, CONTRIBUTORS, AND REVIEWERS

AUTHORS

John Lipscomb, CoLead, U.S. EPA, Office of Research and Development, Cincinnati, OH 45268

Anna Lowit, CoLead, U.S. EPA, Office of Pesticide Programs, Arlington, VA 22202

Brenda Foos, U.S. EPA, Office of Children's Health Protection, Washington, DC 20460

Audrey Galizia, U.S. EPA, Office of Research and Development, Edison, NJ 08837

Elaina Kenyon, U.S. EPA, Office of Research and Development, RTP, NC 27711

Ginger Moser, U.S. EPA, Office of Research and Development, RTP, NC 27711

Rita Schoeny, U.S. EPA, Office of Water, Washington, DC 20460

Michael Broder, Science Coordinator/DDEF Project Staff Lead, U.S. EPA, Office of the Science Advisor, Washington, DC 20460

CONTRIBUTORS

Rory Conolly, U.S. EPA, Office of Research and Development, RTP, NC 27711

Deirdre Murphy, U.S. EPA, Office of Air and Radiation, RTP, NC 27711

Bruce Rodan, U.S. EPA, Office of Research and Development (formerly),[1] Washington, DC 20460

[1]Currently at the Office of Science and Technology Policy, Washington, DC 20460.

EXTERNAL PEER REVIEWERS

Peter J. Boogaard, Senior Toxicologist, Shell International, Grasweg 31, 1031 HW Amsterdam, The Netherlands

Harvey J. Clewell, III, Senior Investigator and Director, Center for Human Health Assessment, The Hamner Institutes for Health Science, Research Triangle Park, NC 27709

Michael L. Dourson, President, Toxicology Excellence for Risk Assessment, Cincinnati, OH 45211

David W. Gaylor, Independent Consultant, Gaylor and Associates, LLC, Eureka Springs, AR 72631

M.E. (Bette) Meek, Associate Director, Chemical Risk Assessment, McLaughlin Institute for Population Health Risk Assessment, University of Ottawa, Ottawa, OH K1N 6N5 Canada

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EXECUTIVE SUMMARY

The mission of the U.S. Environmental Protection Agency ("EPA" or "Agency") is to protect human health and the environment. The statutes under which EPA functions require the Agency to identify substances in the environment that may elicit a response, and determine the potential for harmful effects from exposure to those substances. Risk assessment is an analytical process routinely used by EPA to inform decisions on managing risks to human health and the environment (U.S. EPA, 2012). Because no risk assessment is definitive, uncertainties must be handled in a manner that is both consistent with EPA's policies and responsive to the needs of decision makers (U.S. EPA, 2004a). It is a common practice to resort to default assumptions (including values) to allow the assessment to proceed when data are limited. This document is consistent with the recommendations in *Science and Decisions: Advancing Risk Assessment* (NRC, 2009) as it describes the process for developing scientifically supportable values to account for inter- and intraspecies extrapolation.

Among the default values most commonly used in human health risk assessment are those used to extrapolate toxicity data derived from animal models to humans and those that account for human variability. This document provides guidance to risk assessors who are well versed in chemical dosimetry and/or studies of tissue responses on methods used to account for the differences between the model species and the average human (interspecies variation), and for variation in the human population (intraspecies variation). Moving from the established default values for inter- and intraspecies extrapolation to empirically derived values addresses the recommendations in *Science and Decisions: Advancing Risk Assessment* (NRC, 2009) to "…continue and expand use of the best, most current science to support and revise default assumptions." The Institute of Medicine (IOM) report *Environmental Decisions in the Face of Uncertainty* concluded "… if enough scientific information exists about the differences in the metabolism or mode of action of a chemical in animals versus in humans, then scientifically derived extrapolation factors can be used rather than the defaults." The IOM report goes on to say about data-derived values, "If those factors more accurately reflect the differences between animals and humans than default adjustment factors, the use of such data-derived extrapolation factors would decrease the uncertainty in the risk assessment" (IOM, 2013).

The goals of data-derived extrapolation factors (DDEFs) are to maximize the use of available data and improve the scientific support for a risk assessment. A DDEF approach is an accepted approach for deriving reference concentrations (RfCs), reference doses (RfDs), or counterpart values and is consistent with existing Agency guidance. This guidance presents the Agency's approach to identifying, justifying, and employing quantitatively useful data to develop nondefault values for inter- and intraspecies extrapolation. Moreover, this guidance will

aid risk assessors and researchers in identifying data gaps and developing informative experiments to yield quantitatively valuable data.

DDEF values are applicable in the derivation of RfCs and RfDs, or other relevant values or metrics (e.g., hazard index, margins of exposure). This guidance describes the process for identifying pertinent data useful for quantifying inter- and intraspecies differences to serve as the basis for empirically determined DDEFs. When using DDEFs, inter- and intraspecies extrapolation factors are divided into two components representing toxicokinetic (TK; amount of agent reaching the target tissue) variability and toxicodynamic (TD; dose at which the target tissue responds to the agent) variability. Key considerations include identifying an adverse health outcome, a measurable biological event associated with that adverse health outcome, and the concentration of the toxicant associated with the development of the biological event. Interspecies TK variability is quantified based on the external exposure that produces the same tissue concentration in animals and in humans. Intraspecies TK variability is defined as differences in tissue concentration attained from the same human external exposure (dose). TD variability is quantified on the basis of differences in the tissue or *in vitro* concentration that produce the same response between animals and humans or among humans.

1. INTRODUCTION

1.1. BACKGROUND

Risk assessment is an analytical process used by the U.S. Environmental Protection Agency ("EPA" or "Agency") to inform decisions on managing risks to human health and the environment (U.S. EPA, 2012). No risk assessment can reflect risk with absolute certainty, so it is important that uncertainties be accounted for in a predictable, scientifically defensible manner that is both consistent with EPA's policies and responsive to the needs of decision makers (U.S. EPA, 2004a). The risk assessment process involves decreasing uncertainty in estimates whenever possible, defining uncertainty and variability in estimates, and quantifying the uncertainty when feasible. In deriving reference concentrations (RfCs) and reference doses (RfDs), the Agency has historically used default uncertainty factors (UFs) to compensate for a lack of information (U.S. EPA, 2002b). As science has advanced, however, there has been a growing effort to increase reliance on available data to modify the values for these UFs (IPCS, 2005). The default UFs were developed to address data gaps in the development of RfDs and RfCs, but when appropriate data are available for an assessment, those data are given precedence over standard default values (U.S. EPA, 2004a). This guidance describes an approach for identifying and using pertinent information for developing data-derived extrapolation factors (DDEFs) for the purposes of developing RfDs, RfCs, or related metrics/approaches (e.g., hazard index, margin of exposure).

It is common to use default values and processes in risk assessments to compensate for the absence of data. 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). In its report *Science and Judgment in Risk Assessment* (NRC, 1994), the NRC supported EPA's use of defaults as a reasonable way to consider uncertainty. The report stated that EPA should have principles for choosing default options and for judging when and how to depart from them. Specifically, the report recognized that EPA uses default assumptions (e.g., UF) and indicated that criteria for their use should be clearly articulated in situations in which "the chemical and/or site-specific data are unavailable." In the report *Science and Decisions: Advancing Risk Assessment* (NRC, 2009), the NRC recommended that "EPA should develop clear, general standards for the level of evidence needed to justify the use of alternative assumptions in place of defaults." The current document is responsive to the recommendations in *Science and Decisions: Advancing Risk Assessment* because it describes the process for developing scientifically supportable values to account for inter- and intraspecies extrapolation.

While risk assessors have generally tried to make maximum use of available data, the shift away from standard default assumptions as the starting point in risk assessment was formalized as EPA science policy with the publication of the 2005 *Guidelines for Carcinogen Risk Assessment* [or "*Cancer Guidelines*"; (U.S. EPA, 2005)]. The *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 needed to address uncertainty or the absence of critical information." Applying the available and sufficient data to avoid default UF values will improve the scientific basis of risk assessments when data are sufficient for refining UFs (IOM, 2013). In cases where data are not sufficient and default approaches are used, hazard and risk characterizations will be improved because data needs can be more clearly articulated and potentially met in the future (Bogdanffy et al., 2001; Meek, 2001; Meek et al., 2001; Murray and Andersen, 2001).

Extrapolation is most scientifically robust when data are first evaluated before using defaults. However, with a multitude of types of data, analyses, and risk assessments, as well as the diversity of needs of decision makers, it is neither possible nor desirable to specify step-by-step criteria for decisions to invoke a default option. Some risk assessments may be limited by constraints of data, time, and/or resources. Other risk assessments may require only screening-level evaluations; in these cases, the risk assessor may be more likely to resort to one or more default assumptions. On the other hand, risk assessments used to support significant risk management decisions will often benefit from a more comprehensive approach. In general, the level of effort applied in a particular assessment should be related to the needs of decision makers, as determined through planning and scoping for that assessment (U.S. EPA, 2014).

1.2. PURPOSE AND SCOPE

Efforts by the United States and international communities have improved the scientific basis for human health risk assessments by increasing the use of mechanistic and kinetic data. For example, the *Cancer Guidelines* (U.S. EPA, 2005) emphasize the use of mode-of-action (MOA) information in characterizing potential health effects of exposure to environmental agents. International efforts, including those by the International Life Sciences Institute and the World Health Organization (WHO)'s International Programme on Chemical Safety (IPCS), have developed frameworks for evaluating animal data to determine the human relevance of described MOAs (Boobis et al., 2008; Seed et al., 2005; Sonich-Mullin et al., 2001).[2] These documents

[2]Use of the term adverse outcome pathway (AOP) has become common. AOPs and MOAs are similar in that they identify an initiating event and the important biological steps associated with different levels of biological organization leading to an adverse health outcome.

guide the qualitative and quantitative evaluation of the relevance of a particular animal MOA in humans and discuss the use of *in vivo* and *in vitro* data when considering animal-to-human extrapolation. The 2005 *Cancer Guidelines* (U.S. EPA, 2005) and other documents such as IPCS's chemical-specific adjustment factors (CSAFs) guidance (IPCS, 2005), the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), and *An Examination of EPA Risk Assessment Principles and Practices: Staff Paper Prepared for the U.S. EPA by Members of the Risk Assessment Task Force* (U.S. EPA, 2004a) also encourage the use of sophisticated models like physiologically based pharmacokinetic (PBPK) and biologically based dose-response (BBDR) models in interspecies extrapolation.

This guidance deals specifically with the development and use of DDEFs in the calculation of RfDs, RfCs, and other relevant approaches (e.g., margin of exposure) to assessing risk. The goal of DDEFs is to maximize the use of available data and improve the scientific support for a risk assessment. The processes described herein have benefited from ongoing discussions in the scientific community regarding the need to refine the default 10-fold UFs historically used in deriving safety estimates (e.g., RfDs, minimal risk levels, and acceptable daily intakes). Finalized in 2005 (IPCS, 2005), the WHO CSAF guidance describes approaches for using data to refine inter- and intraspecies default UFs. *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* is based largely on analyses by Renwick (1993, 1991) and Renwick and Lazarus (1998), which describe a data-derived approach that assigns values for toxicokinetic (TK) and toxicodynamic (TD) differences as components within an established 10 × 10 framework for inter- and intraspecies extrapolation. DDEFs are similar in concept to the CSAFs in that the factors for interspecies and intraspecies extrapolation are subdivided into TK and TD components, and kinetic and mechanistic data are used to derive refined inter- or intraspecies extrapolation factors.

LINKING TOXICOKINETICS AND TOXICODYNAMICS

Interactions between the toxicologically active chemical moiety and the cellular receptor are responsible for producing an adverse response. Therefore, this guidance presents a single methodology to quantify differences in target tissue concentrations of toxicants (toxicokinetics) and differences in target tissue responses to toxicants (toxicodynamics) to avoid reliance on default values for inter- and intraspecies uncertainty factors when data are available.

The Appendix to this document contains case study examples taken from EPA's Integrated Risk Information System (IRIS) and from EPA Program Office records. These case studies present the application of principles contained in this document to data and modeling studies for actual chemicals and should serve as instructional aides.

Issues related to the derivation and use of DDEFs to avoid default UFs for intraspecies (human) variability and interspecies variability are the focus of this guidance document. Thus, concepts beyond the scope of this guidance are not discussed in detail here; they include approaches for selecting critical effects, establishing key events in an MOA analysis,[3] deriving points of departure (PODs), performing benchmark dose analysis, and developing and evaluating PBPK and BBDR models. In addition, no discussion is included on factors that have been used for other areas of uncertainty or variability (e.g., duration, database deficiencies, or lack of a no-observed-adverse-effect level).[4]

Finally, this document is written for toxicologists and risk assessors, and the methods described here should be conducted by or in conjunction with scientists with the appropriate level of expertise.

[3]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 2.3 for further information on use of MOAs in developing DDEFs.

[4]The Food Quality Protection Act mandates the use of a presumptive 10-fold factor in risk assessments performed for establishing pesticide tolerances as part of pesticide registration for the protection of infants and children in addition to inter- and intraspecies factors. This factor can only be modified based upon reliable data.

2. TECHNICAL CONCEPTS AND PRINCIPLES FOR DATA-DERIVED EXTRAPOLATION FACTORS

2.1. BACKGROUND

EPA has developed several methodologies to guide and refine the approach to estimate reference values for human exposures. This approach comprises several steps that include inter- and intraspecies extrapolation, in which UFs based on default assumptions may be required to account for inherent uncertainties and variability (U.S. EPA, 2011, 2002b; Bogdanffy and Jarabek, 1995; U.S. EPA, 1994; 1993, see Figure 1). This guidance describes an approach to performing inter- and intraspecies extrapolations based on the use of the best available science

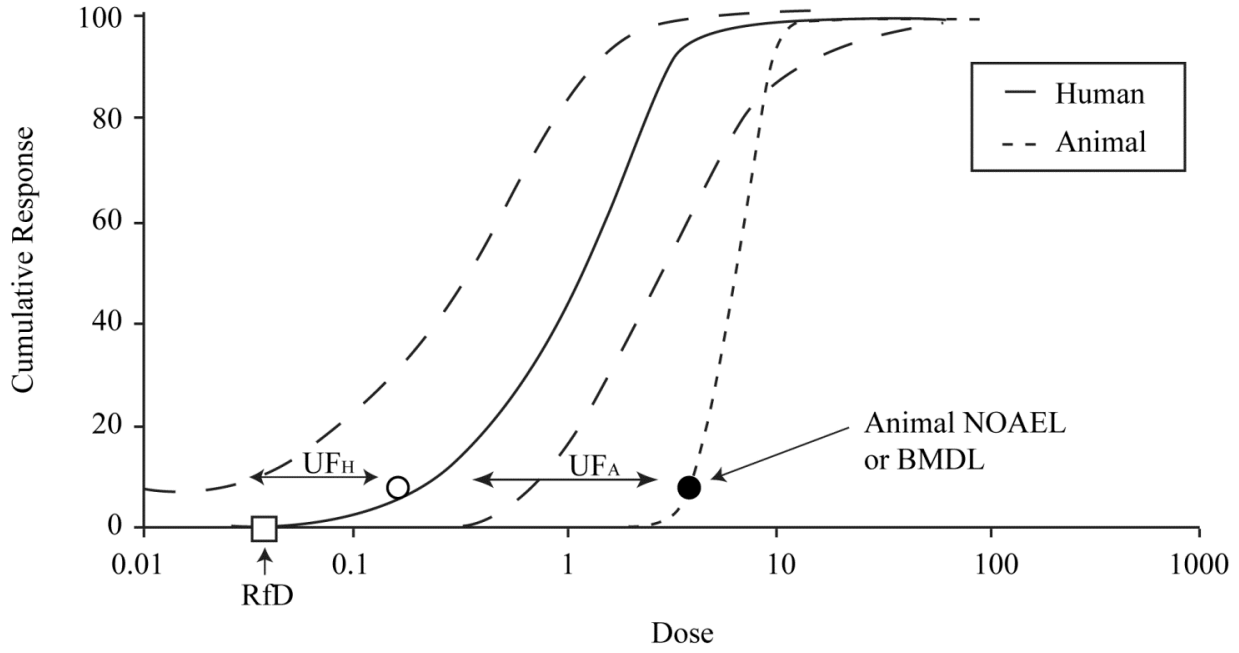

Figure 1. Derivation of reference dose/reference concentration using uncertainty factors. This figure depicts the extrapolation of the dose-response relationship between and among species. The POD (filled circle) for the animal dose-response relationship (dotted line) is extrapolated to humans (solid line) through application of the interspecies uncertainty factor (UF_A), which is "applied to account for the extrapolation of laboratory animal data to humans, and it generally is presumed to include both TK and TD aspects." Here, per long-standing guidance, the dose at the animal POD associated with a predetermined level of response is extrapolated using UF_A to a measure of dose assumed to represent the same level of response (open circle) for a central tendency member of the general human population (solid line) (U.S. EPA, 1993). Dashed lines surrounding the solid line represent confidence bounds for human variability. Note that while the dose is extrapolated to other values, the response level remains fixed both between animals and humans, as well as within the human population.

and data. The rationale for choosing an extrapolation factor value should be presented transparently, include a full discussion of the perceived strengths and limitations of the data and describe the impact of science policy considerations include relevant science policy choices or implications

In the context of the methodologies for the derivation of RfDs, RfCs, and other relevant metrics, DDEFs are intended to address, as needed, inter- and intraspecies extrapolation of the POD from experimental data to an estimate for the sensitive human population or life stage. DDEFs are developed from data on inter- or intraspecies differences. DDEFs may consider both TK and TD properties. These factors can be derived for a single agent or chemical, for a class of chemicals with shared chemical or toxicological properties, or for a group of chemicals that share a mode or mechanism of action or TK characteristics. As described below, DDEFs can be calculated using sophisticated TD or TK models or can be calculated as ratios using key kinetic or dynamic data. With regard to interspecies extrapolation, EPA currently recognizes a hierarchy of approaches ranging from the preferred approach using PBPK modeling (U.S. EPA, 2011, 2006a, 1994) to default approaches when data do not support a more chemical-specific approach. "The intraspecies uncertainty factor (UF_H) is applied to account for variations in susceptibility within the human population (interhuman variability) and the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure-response relationship in the groups of the human population that are most sensitive to the health hazards of the chemical being assessed" (U.S. EPA, 2002b). The default value for UF_H is 10-fold; the default value for interspecies uncertainty factor (UF_A) is apportioned into a TD component valued at one-half order of magnitude and a TK component addressed via default inhalation dosimetry methods (U.S. EPA, 1994) or body-weight scaling for orally encountered compounds (U.S. EPA, 2011). DDEFs fall within this hierarchical range of approaches.

CHEMICAL-SPECIFIC DATA

When deriving an RfD/RfC from animal data, and in the absence of information to the contrary, humans are assumed to be more sensitive to the toxic effect of chemicals than are test animal species. Humans also demonstrate population variability in response. These differences in sensitivity between species and among humans are captured in two uncertainty factors: interspecies (UF_A) and intraspecies (UF_H), respectively (U.S. EPA, 2002b). The default values for these UFs are based on our understanding and interpretation of data for a limited number of chemicals. With data relevant to the chemical of interest, DDEF may be used instead of the default values, thus increasing the confidence in the assessment.

Avoiding default assumptions with DDEFs begins with an evaluation of the strengths of the available data. Using *in vitro* data in risk assessment, as advocated in *Toxicity Testing in the 21st Century* (NRC, 2007), offers some distinct advantages over *in vivo* studies. While data derived in whole animal bioassays offer some value in deriving DDEF values, the data are accompanied by limitations. Given that humans and test animal species may differ in terms of

both dosimetry and innate sensitivity, response data from whole animal bioassays offer little opportunity to separate the TK and TD components of uncertainty. *In vitro* systems offer some advantages in that the influence of TK can be well controlled and response data can be well characterized, largely due to the avoidance of experimental constraints (e.g., less restrictive constraints on resources in areas like the number of doses/concentrations for testing). *In vitro* systems also offer some distinct advantages in studying both metabolism and response development in the human species because the ethical considerations of exposure are substantially lessened. Regardless, *in vitro* data require interpretation in the context of the intact mammalian system. For example, when *in vitro* data are derived from preparations representing only a fraction of the total biology of the cell (e.g., microsomal protein), care should be taken to ensure that the measured TD event (e.g., protein binding) or TK outcome (e.g., formation of an/the active metabolite) accurately reflects the biology of the *in vivo* effect. Regardless of the system evaluated (*in vitro* or *in vivo*), determinations regarding the strengths of the relevant data require careful consideration and characterization.

2.1.1. Uncertainty Factors Compared to Data-Derived Extrapolation Factors

DDEF values are not UFs, *per se*. UFs incorporate both extrapolation components that address variability (heterogeneity between species or within a population) and components that address uncertainty (i.e., lack of knowledge); (U.S. EPA, 2002b; Dourson et al., 1996; Dourson and Stara, 1983), whereas DDEFs focus on variability. Additionally, interspecies and intraspecies UFs are values based on general assumptions, whereas data-derived values are empirically determined based on chemical-specific data.

Thus, DDEF values are more precise and accurate than default UF values, but the values for the DDEF components may sometimes be similar to default values for UFs. Regardless of any similarity to default UF values, developing a DDEF quantifies variability and reduces uncertainty, carrying with it a change in nomenclature (IOM, 2013).

UNCERTAINTY AND VARIABILITY EXPLAINED

Variability refers to true heterogeneity or diversity. This may be due to differences in exposure as well as differences in response. Those inherent differences are referred to as variability. Differences among individuals in a population are referred to as interindividual variability, while differences for one individual over time are referred to as intraindividual variability. DDEF values quantify variability on the basis of chemical specific information.

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

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

Evaluation of the sources and magnitude of uncertainty accompanying DDEF values is informative (U.S. EPA, 2005, 2001c, 1997a, b), and quantitative uncertainty analyses may be undertaken, but such analyses are not presented in this guidance. When quantitative approaches are not feasible, qualitative uncertainty analyses may be developed. Furthermore, as discussed in the 2005 *Cancer Guidelines*, "a default option may be invoked if needed to address uncertainty or the absence of critical information."

The use of human response data for the critical effect obviates the need for a UF_A. The richness of a human data set may offer additional potential to develop DDEF values for interindividual variability. It may prove difficult to separate the contributions of TK and TD in these data sets without additional data and/or models.

RESPONSE DATA IN HUMANS

For some chemicals, the available data describing adverse effects in humans are suitable for dose-response analysis. When the POD is derived from studies with humans, the need for a UF_A is obviated, and the value for UF_A is set to 1.

The capability to develop a DDEF value depends on the availability and suitability of experimental data and/or predictions from reliable models (see Section 2.2.5). Once data sets are evaluated to justify their basis for a quantitative reliance, three primary sets of information are required to develop a DDEF value:

- Sufficient information on the MOA, such as understanding of the major steps leading from exposure to adverse outcome, including identification of the toxicologically active chemical species;
- Identification of the target tissues or organs; and
- Availability of information to determine whether an instantaneous (i.e., maximum concentration [C_{max}]) or a time-normalized (i.e., clearance [Cl] or area-under-the-curve, [AUC]) measure of exposure is the more appropriate basis for tissue response.

2.1.2. Sensitivity and Susceptibility in the Context of Data-Derived Extrapolation Factors

For the purpose of this DDEF guidance, the terms susceptibility and sensitivity are used interchangeably and defined as an increased response to a given exposure. [Note the term *susceptible* is also used to describe *sensitive* or *vulnerable* populations or life stages. These terms have varying definitions within EPA documents and are used interchangeably. No convention for use of the terms sensitivity, susceptibility, or vulnerably is widely accepted (U.S. EPA, 2004a). The term, vulnerability, is not used in this document.] Susceptibility in the human population may be due to life stage, health status or disease state, genetic disposition, exposure, or other factors. Therefore, with respect to intraspecies variability, it is important to consider the

factors that lead some individuals or groups to be more sensitive than others. Humans respond differently to chemical exposures based on several factors that can be exogenous and/or intrinsic. Exogenous factors relate to exposure conditions such as chemical concentration/external dose, media, pathway, or duration. Physiological, anatomical, and biochemical parameters are intrinsic factors that may also be the basis for differential susceptibility among the population and at different life stages. Intrinsic factors can mediate sensitivity by influencing the target tissue concentrations of the chemical inside the body (TK; see Section 3.3.1.1) or by modulating an increased responsiveness of the tissues to the toxicologically active chemical species (TD; see Section 4.3.1.1). For some chemicals, data may be sufficient to identify one or more sensitive populations or life stages.

Life stage is a key consideration in susceptibility. Developing organisms (e.g., fetus, infant) can be more sensitive for several reasons, some of which include a higher body mass-adjusted exposure and the potential for increased sensitivity of rapidly growing tissues. Critical windows of development, and therefore windows of sensitivity, occur at different times for various tissues, organs, and systems; therefore, considering susceptibility to more than one critical effect may require consideration of more than one life stage. The aged may also represent a sensitive life stage.

Toxicity (response) data from the sensitive life stage may be used directly to identify the POD. In other cases, TK or TD data may be used in derivation of DDEFs to extrapolate POD values, for example from the average adult to the sensitive life stage. Because every human being goes through developmental life stages, sensitive life stages are not a population *per se*, but sensitive life stages do need to be considered explicitly in the risk assessment when sufficient data are available. For purposes of this guidance, life stages are considered among the multiple potentially sensitive populations.

With respect to TK, sensitivity is the result of higher tissue concentrations being attained at a fixed dose. Elevated tissue concentrations may be the result of an increased distribution to tissues or a decreased elimination from tissues. Regarding measures of tissue concentrations, maximum concentration (C_{max}) and AUC are suitable measures, and sensitive individuals or sensitive populations will be those at or near the upper tail of the population distribution. With respect to measures of the removal of toxicant, measures of Cl are suitable, and sensitive individuals or sensitive populations will be at the lower tail of the population distribution. Sensitive individuals or sensitive populations will be those in which a predetermined level of response will be reached at lower tissue (or *in vitro*) concentrations. A quantification of DDEF values based on measures obtained from those deemed sensitive and those representing the generally responsive portion of the population are described later (TK in Section 3.3.2.2; TD in Section 4.3.2.2).

The development and evaluation of experimental data and models describing TK and TD are likely to lead to an improved understanding of population sensitivities and thus population variability, as well provide a means for quantitation. Understanding population sensitivity and characterization of population variability will improve the scientific basis for human health risk assessment.

2.2. DERIVING AND APPLYING DATA-DERIVED EXTRAPOLATION FACTORS

The foundation of DDEFs is the concept that the toxicity of a particular agent is due to a combination of both TK and TD factors, and that those factors can be quantified in animals and humans. For purposes of this guidance, TK is defined as the determination and quantification of the time course and dose dependency of absorption, distribution, metabolism, and excretion (ADME) of chemicals (sometimes referred to as pharmacokinetics) of the chemical agent, while TD is defined as the determination and quantification of the sequence of events at the cellular and molecular levels leading to a toxic response. TK and TD share a common point—each is concerned with the concentration of the toxicologically active chemical species in the target tissue. As such, it can be difficult to establish a clear separation between TK and TD because the processes leading to biological responses include aspects of both—including interactions between TK and TD processes.

SUBDIVIDING UF_A AND UF_H

The response to toxicants is based broadly on two functions: target tissue exposure (i.e., TK) and innate sensitivity to the insult that modulates the type and severity of the response (i.e., TD). Thus, both UF_A and UF_H have been divided into TK and TD components. This distinction was described in the RfC guidance for inhaled substances (U.S. EPA, 1994). The subdivision of UF_H has been applied in several assessments described in Appendix A. The subdivision of the UFs provides the framework for the quantitative inclusion of TK and TD data sets in inter- and intraspecies extrapolation.

Extrapolation from animals to humans and within the human population can be accomplished by one of several approaches ranging from the use of sophisticated BBDR models to the calculation of relatively simple ratios using TK or TD data describing critical factors in inter- or intraspecies extrapolation. The following text describes the approaches for calculating the different DDEF values. In the absence of data for performing sophisticated modeling or for deriving DDEF values, default approaches for toxicokinetics are used, but no such approaches for toxicodynamics are yet available.

Four DDEFs can be calculated given sufficient information. Two extrapolation factors are for interspecies extrapolation from animal data to humans (EF_A): (1) extrapolation factor covering interspecies toxicokinetics (EF_{AK}) is calculated to account for TK variability, while (2) extrapolation factor covering interspecies toxicodynamics (EF_{AD}) accounts for TD variability. Likewise, there are two extrapolation factors dealing with variability within the human population (EF_H): (1) extrapolation factor covering intraspecies toxicokinetics (EF_{HK}) for TK and

(2) extrapolation factor covering intraspecies toxicodynamics (EF_{HD}) for TD. Table 1 provides example equations for calculating these DDEFs. Section 3 describes how to calculate and when to use TK factors for interspecies (see Section 3.2) and intraspecies (see Section 3.3) extrapolation. Section 4 describes how to calculate and when to use TD factors for both interspecies (see Section 4.2) and intraspecies (see Section 4.3) extrapolations. Section 5 describes how to combine EF_{AK}, EF_{AD}, EF_{HK}, and EF_{HD} into the composite UF.

Table 1. Example equations used to derive data-derived extrapolation factors

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

EF_{AK} = interspecies TK extrapolation factor.
D_A = animal external dose (administered or external dose in the test animal species that leads to a level of a toxicologically relevant dose metric at or near the POD).
D_H = human external dose (administered or external dose at the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species).
EF_{AD} = interspecies TD extrapolation factor.
$Concentration_A$ = animal concentration (concentration of the agent in the tissue or *in vitro* in the test animal species corresponding to a level of response near the animal POD).
$Concentration_H$ = human concentration (concentration of the agent in the tissue or *in vitro* in the human corresponding to a level of response near the animal POD).
EF_{HK} = intraspecies TK extrapolation factor.
AUC_{gen} = general human population AUC value (area under the concentration-time curve at a fixed external dose at a measure of central tendency in the entire or general human population).
AUC_{sens} = sensitive human population AUC value (area under the concentration-time curve at a fixed external dose at a percentile of interest for the sensitive human population or representing sensitive individuals among the entire human population).
EF_{HD} = intraspecies TD extrapolation factor.
$Concentration_{gen}$ = general human population concentration (concentration producing the response corresponding to the POD at a measure of central tendency in the general human population).
$Concentration_{sens}$ = sensitive human population concentration (concentration producing the response corresponding to the POD at a percentile of interest for the sensitive human population or representing sensitive individuals among the entire human population).

The benefit of DDEFs is that they maximize the use of available data and improve the overall scientific support for a risk assessment. Figure 2 provides a flowchart of the decision process for the extrapolation used in deriving DDEFs. As described in more detail in Sections 3 and 4, it is important for the human health hazard and/or risk characterizations to include

thorough and transparent discussions of methods and data used to support extrapolation approaches.

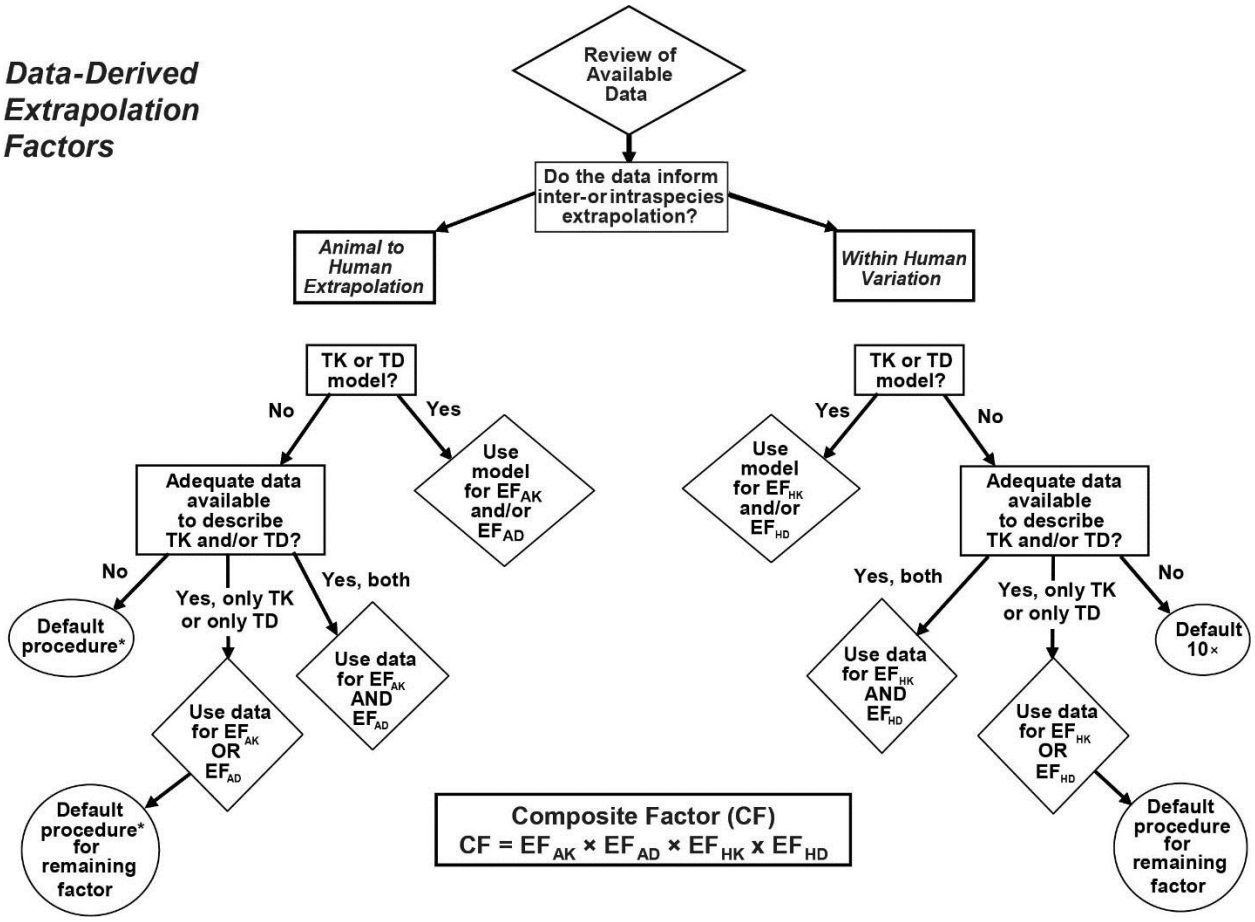

Figure 2. Decision process for data-derived extrapolation factors. The availability of an adequate TK or TD model is considered first, followed by analysis of the availability of adequate data to describe the TK and/or the TD of the chemical. With the availability of an adequate model or data, data-derived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

*For interspecies extrapolation, the default procedure is ¾ body-weight scaling for RfD (U.S. EPA, 2011) and the RfC method (U.S. EPA, 1994) for inhalation to derive a human equivalent dose (HED) and human equivalent concentration (HEC), respectively. When these methods are used in deriving the RfD and RfC, the default interspecies UF is then reduced to a value of 3. The composite factor (CF) accounts for inter- and intraspecies extrapolation and can comprise default or DDEF values for the four extrapolation factor components.

2.2.1. TK and TD Models

TK and TD data and/or models represent the preferred approach to intra- and/or interspecies extrapolation. Models 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 TK and/or MOA of chemical(s). In these TK and TD models, some measure of the internal dose is related to the external dose and response, respectively. When available, BBDR models combine TK and TD modeling, using the measure of internal dose or dose metric to link the TK and TD aspects of the modeling approach (see discussion in Section 4.2.2.2).

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

PBPK models differ from classical compartmental models in that they are composed of compartments with realistic tissue volumes that are linked by blood flow. Other parameters used in these models account for chemical-specific characteristics that can be independently measured in both humans and laboratory animals (usually using *in vitro* techniques); these chemical-specific parameters include tissue solubility (i.e., partition coefficients), binding, and metabolism. These models are used to simulate the relationship between applied (administered) dose and internal dose at the target tissue. PBPK models require more data to develop compared to classical compartmental models, but they are advantageous because they can be used for extrapolation [i.e., across dose range, among animal species, between routes of exposure, and across exposure scenarios (U.S. EPA, 2006a; Krishnan and Andersen, 1994)].

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

extrapolation. However, with an adequate understanding of the nature of the response and sufficient empirical data describing the dose-response function in relevant species or populations, a fully developed TD model may not be required to develop a DDEF.

2.2.2. Use of Ratios to Calculate Data-Derived Extrapolation Factor

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

TARGET TISSUE CONCENTRATIONS IN DDEF CALCULATIONS

DDEF values are based on an understanding of target tissue concentrations, rather than relying solely on external concentrations or effective doses. For TK (see Section 3), interspecies differences are calculated as differences in external (administered) dose resulting in the same target tissue concentration, and intraspecies differences are calculated as differences in internal concentrations resulting from the same external dose or exposure. TD differences (see Section 4) are calculated as differences in target tissue concentrations resulting in the same response level.

In general, *interspecies* extrapolation involves calculating a ratio of animal data (for a kinetic or dynamic parameter) to human data for a kinetic or dynamic parameter. Similarly, for *intraspecies* extrapolation from the general (average) human population to the sensitive population, a ratio is calculated using data from the sensitive population and that for the central tendency of the general population. Data to derive the TK factors may come from *in vivo* or *in vitro* studies. For TD, in general, interspecies extrapolation may have its basis in data from *in vivo* studies but may often be accomplished with *in vitro* data in a relevant tissue. When adequate data on toxic effects are available in humans, these data may be considered when identifying a POD, eliminating the need for the interspecies extrapolation. Otherwise, the human information can be used to inform an interspecies factor when the POD is derived from animals, allowing a quantitation of UF_A components, rather than relying on default values.

SENSITIVITY

In the absence of data to the contrary, it is assumed that humans will exhibit response in the same tissues as in test species. When humans are more toxicodynamically sensitive than animals, humans will demonstrate the same level of response, but at lower tissue concentrations. If the same dose results in higher observed or predicted AUC or C_{max} values, or lower clearance values in humans than in animals, then humans are more (toxicokinetically) sensitive and EF_{AK} will be greater than 1.

Intraspecies susceptibility may be based on differences in TK or TD. Sensitive populations will demonstrate higher tissue concentrations at the same dose (TK), or the same type and level of response at lower tissue concentrations (TD).

For interspecies extrapolation, it is preferred that the ratio be based on data at or near the POD. When sufficient data are available, interspecies DDEF values should be calculated for a

range of doses near the POD because the shape of the dose-response curve can vary among species. Metabolism and kinetic properties can vary across doses, particularly in the higher dose ranges; thus, developing multiple estimates of DDEF values at or near the POD helps avoid potential uncertainty in the DDEF estimate that may be introduced by nonlinearity in kinetic properties. Moreover, evaluating a range of PODs takes into account the dependence of the DDEFs on the POD selected. One way to address this is to calculate interspecies DDEF values at multiple doses in the range of the POD to demonstrate the stability of the DDEF value. Likewise, when the POD is expressed with a confidence bound, some effort can be taken to include this range of POD values when developing DDEF values. The interspecies DDEF values should be derived using an estimate of central tendency, such as the mean, median, or mode, depending on the characteristics of the data. In contrast, when calculating intraspecies DDEF values, the ratio includes a measure of central tendency of the general population and percentiles of the distribution representing those potentially sensitive (see Section 2.1.2). As the needs of risk managers and decision makers vary, it is recommended that the risk assessor consult with the risk manager or decision maker to determine the risk assessment objectives (U.S. EPA, 2014). A range of percentiles may be useful and thus evaluated and their corresponding DDEFs be reported in the human health hazard and risk characterizations.

DATA FROM SENSITIVE POPULATIONS

The POD may be determined in test animals, in the general human population, or in susceptible human populations. For some well-studied chemicals (e.g., nitrate, fluoride), dose-response data from the sensitive human population may be available. When these data are sufficient to identify a POD in the sensitive population, the issue of human variability has been addressed and the need to apply an intraspecies uncertainty factor (UF_H) is obviated—UF_H is set to a value of 1.

TK ratios (for either interspecies or intraspecies extrapolation) are informed by the MOA and are based on the relevant dose metric, such as AUC and the C_{max}.[5] Other metrics (e.g., AUC above a threshold) may be used if supported by the data or if relevant for a particular chemical or MOA. For toxicants that bind covalently or cause irreversible damage, especially as a consequence of subchronic or chronic exposure, an integrated measure

AREA UNDER THE CURVE, CLEARANCE, AND HALF LIFE

Area under the curve (AUC = (μg/ml) × hr) is related to total dose; clearance (Cl = ml/min per kg body weight) is independent of dose and inversely related to AUC. Half-life is not an acceptable basis for DDEF calculation because it is related to neither body weight nor volume of distribution. When clearance decreases, AUC values increase; when clearance increases, AUC values decrease. When a chemical does not induce or inhibit its own metabolism or clearance, AUC or clearance values after a single dose, when extrapolated to infinity, may prove a suitable alternative basis for DDEF calculation.

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

of dose over time such as AUC is preferable (O'Flaherty, 1989). In the case of effects occurring as a consequence of acute exposure or when toxicity is related to exceeding an internal concentration threshold, C_{max} may be more appropriate (Barton, 2005; Boyes et al., 2005). When data on chemical-specific AUC, C_{max}, or Cl are not available, a chemical-related physiological parameter (e.g., renal glomerular filtration rate) that is critical to the onset of toxicity or to the MOA may be used.

2.2.3. Default Methods for the Derivation of Reference Concentrations, Reference Doses, and Other Relevant Metrics

In accordance with the hierarchy of approaches, when available agent-specific data are supportive of DDEF derivation from use of models or from ratios, a data-derived approach is preferred over using the default RfC approach or ¾ body-weight scaling. When deriving reference values (or counterpart values) from an animal POD in the absence of applicable TK and/or TD data in animals and humans, a default uncertainty factor value is applied unless it can be concluded that the test species is equally or more susceptible than humans (U.S. EPA (2002b).

The default approach for the inhalation exposure route (i.e., RfC) involves applying both a categorical dosimetric adjustment factor to account for species differences in tissue exposure (i.e., TK) and a residual UF of a value of one-half order of magnitude, that is generally described as covering TD (U.S. EPA, 1994). The dosimetric adjustments are based on the following:

- Anatomical and physiological 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 dose, according to body weight to the ¾ power ($BW^{3/4}$), and applying a UF of one-half order of magnitude to account for residual uncertainty (U.S. EPA, 2011).

After default adjustment between species, the residual UF associated with either route (oral or inhalation) has a default value of one-half order of magnitude, which may be modified based on available data (U.S. EPA, 2011, 1994).

2.2.4. Qualitative Considerations

Although in some cases data may be insufficient for a quantitative estimate of a DDEF, there may still be information to support a UF different from the default. For example, there may be qualitative evidence based on an MOA that humans are less sensitive than animals or that

certain groups are more sensitive than the central tendency of the general population. In these cases, where only qualitative data are available, a thorough weight-of-evidence analysis can be considered with the hazard characterization to discuss the derivation of the DDEF along with associated uncertainties in the available database.

2.2.5. Information Quality

Before conducting a DDEF analysis, it is recommended that the risk assessor perform a critical evaluation of all data that may be used to support the development of DDEFs. As an important step in the process, it is advised that data providing qualitative support for the MOA and choice of dose metric, as well as data used in the quantitative derivation of the DDEF itself, be examined. Documentation of the types of literature and data evaluated and a summary of the strengths and weaknesses of data sets should be provided. This will instill confidence in the selection of data chosen as the basis for DDEF derivation, as well as provide an increased understanding of the rationale for any dismissed data. Supporting studies can be evaluated using EPA guidance documents, including the 2005 *Cancer Guidelines*, as well as earlier guidelines specific to neurotoxic, reproductive, and developmental endpoints (U.S. EPA, 2005, 1998, 1996, 1991). In addition, general principles outlined in the EPA information quality guidelines are consulted when critically evaluating data used to support the development and application of DDEF values (U.S. EPA, 2002a).

Use of secondary data sources is one area for particular consideration. Examples of secondary data sources include compilations of pharmacokinetic parameters (e.g., Brown et al., 1997) and studies cited and summarized in toxicity profiles and review articles. In general, for principal and supporting studies used directly in the derivation of DDEF values, a review of the original literature is recommended. In the case of critical assumptions and data, contradictory results from different studies are best resolved by reviewing the original publications.

Quantitative TK and TD data used in the DDEF-derivation process require particular attention to the appropriateness of the study design, the analytical methodology used, and the statistical analysis of the data. Consideration of appropriate study design extends beyond simply verifying that the methods used were adequate for the goals of the study; it also encompasses consideration of the relevancy of the animal or *in vitro* test system used to derive the DDEF for the endpoint of concern. Relevance can be assessed in both qualitative and quantitative terms. For example, if there is a lack of concordance (i.e., a particular TK or TD process relevant to the endpoint does not occur in the test system), or if physiologically unrealistic conditions are used, or different tissue or cell types are evaluated, then the relevancy of the data may be uncertain. Particular considerations relevant to the use of *in vitro* data are discussed below. Another important factor in terms of relevancy is to consider whether the TK or TD response represents a

uniquely sensitive tissue, process, or population. This decision is a critical determinant in evaluating the use of data to describe intraspecies variability.

2.3. MODE OF ACTION

Information on MOA is important in DDEF derivation, even when a complete understanding of the mechanism is not available. DDEFs for both TK and TD are endpoint driven—that is, they are considered in the context of the toxic endpoints most relevant for purposes of the risk assessment. Understanding the MOA(s) for the agent(s) of interest ensures that the TK or TD parameter used to derive the DDEF will be causally related to the adverse outcome of interest. The key events in MOA are likely to identify important metabolite(s) and can aid in identifying potential life-stage susceptibility, sensitive population groups, and/or species differences. Moreover, data on key events may be used directly to estimate EF_{AK} or EF_{AD}.

In the 2005 *Cancer Guidelines*, EPA describes the MOA evaluation as the critical information that defines the conditions under which a toxicant causes its effect, the relevance of animal data for hazard identification, and the most appropriate approach to low-dose extrapolation. The 2005 *Cancer Guidelines* also presents a framework for evaluating data in support of an MOA determination. Major components of this framework include a description of the hypothesized MOA and a discussion of the experimental support for the hypothesized MOA based on modified Hill criteria (U.S. EPA, 2005) for demonstrating associations in human studies.

The MOA is defined as a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through functional and anatomical changes, and resulting in toxicity. A key event is an empirically observable precursor step that is itself a necessary element of the MOA or is a biologically based marker for such an element. MOA is contrasted with "mechanism of action," which implies a more detailed understanding and description of events, often at the molecular level, than is meant by MOA (U.S. EPA, 2005). As a result of the 2007 NRC report on *Toxicity Testing in the 21st Century* (NRC, 2007), the concept of the adverse outcome pathway (AOP) has been introduced (Ankley et al., 2010). An AOP links a molecular initiating event, or mechanism of action, to progressive levels of biological organization at the individual or population level. As such, this framework is conceptually similar to, but in some cases may be more comprehensive than, MOA and would be particularly useful for derivation of DDEFs.

2.4. USE OF *IN VITRO* DATA

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

- Was the toxicologically active form of the agent studied?
- How directly was the measured response linked to the adverse effect?
- Are the biological samples used in the assays derived from equivalent organs, tissues, cell types, age, stage of development, and sex of the animals/humans in which the target organ toxicity was identified?
- What is the range of variability (e.g., diverse human populations and life stages) that the biological materials cover?[6]
- If the effect occurs or can be measured in several tissues, is the studied tissue or tissue preparation an appropriate surrogate? Or, in situations where the effect is not localized, is the effect consistent across tissues?
- Does the design of the study allow for statistically valid comparisons based on such factors as replicate and sample size?
- Was chemical uptake considered when the chemical was applied to the samples so as to give comparable intracellular concentrations across tissues?
- Were similar tissues or samples evaluated across species?
- Do the concentrations in the *in vitro* studies allow for comparison with *in vivo* conditions?

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

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

2.5. MULTIPLE POTENTIAL CRITICAL EFFECTS

For some toxicants, multiple adverse effects may be identified during hazard identification; these may occur at similar doses or exposures and may be the result of a common similar, a dissimilar, or an unknown MOA. It is also possible that the uncertainty and/or variability associated with the TK and/or TD of each of the several adverse effects may differ, resulting in different DDEFs or the retention of default values for UFs, which may lead to differences in dose extrapolation and different reference values. One explanation is that risk assessors may be more certain about inter- and intraspecies differences for one effect versus another. For that reason, the results generated for the multiple responding tissues/organs can be presented for comparison (e.g., in a table that is accompanied by a discussion of the methods used), particularly if multiple MOAs are operational or unknown. It is important not to mix DDEFs derived for one tissue or one MOA with DDEFs (or default UFs) derived from a different tissue unless they can be justified on the basis of the biology of the insult. For example, DDEF values for kidney effects may not apply to liver effects due to innate differences in physiology and biochemistry of the tissues.

DDEF VALUES AND CRITICAL EFFECTS

The PODs for multiple potential critical effects should be combined with their respective default UF or DDEF values to produce an array of potential reference values.

3. DATA-DERIVED EXTRAPOLATION FACTORS BASED ON TOXICOKINETICS

3.1. GENERAL CONSIDERATIONS

TK is concerned with the ADME of chemicals, with an emphasis on the exposure of the biologically active chemical species to the target tissue of interest. Data on tissue concentrations of toxicants or clearance rates of toxicant removal serve as the basis for deriving DDEF values for TK components. This section provides a discussion of factors common to the derivation of both inter- and intraspecies values to account for TK variability. Given the UF_A and intraspecies uncertainty factor (UF_H) framework for uncertainty and extrapolation, there are three generally identifiable points bounding inter- and intraspecies extrapolation: (1) the animal model, (2) the general human population, and (3) sensitive populations or life stages. When a sensitive population(s) or life stage(s) has been identified, and when TK data in animals and the sensitive population(s) are available, these data may be employed to develop a DDEF value for TK that combines both inter- and intraspecies extrapolation. In this case, the DDEF value represents both EF_{AK} and EF_{HK}. Since this situation is not common, this section addresses inter- and intraspecies extrapolations separately.

Data on the quantitative TK differences between animals and humans are used for EF_{AK}. TK differences among the human population are used for the EF_{HK}. Thus, the factor EF_{AK} accounts for extrapolation from laboratory animals to the general human population. EF_{HK} accounts for the variation due to TK in the exposure associated with the critical effect between the human population group represented by the dose-response assessment and sensitive human individuals or populations. Developing a DDEF for TK requires knowledge about the relationship between external dose and internal (target tissue) concentrations. This information can come from studies in which tissue concentrations are measured or predicted, in which both types of data are recorded, or from adequate TK models, which expand the range of confidence from that of the empirical observations. TK models, especially PBPK models, represent an important tool through which *in vitro* observations can be interpreted in the context of the intact system. As such, they represent an advantageous means to evaluate the impact of studies (especially those using human tissues) conducted *in vitro*.

The TK portion of each factor (EF_{AK}, EF_{HK}) is combined with the corresponding TD factors to assemble the composite UF (see Section 5). When the data are not sufficient to derive a DDEF for TK, other approaches can be considered for EF_{AK} or EF_{HK}. For example, the RfC approach (U.S. EPA, 1994) describes default procedures for interspecies extrapolation for inhaled substances. Some important questions to address for TK include:

- What is/are the critical effect(s) and POD being used for this assessment?
- Has the toxicologically active chemical moiety been identified?
- What is the MOA, AOP, or mechanism for that toxicity? Have the key events been identified and quantified? Do these key events identify important metabolic steps?
- Are the processes of ADME of the chemical well characterized? If dose-response data are from an animal model, 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 values for this chemical(s)? Have sensitive populations and/or life stages been identified? Are the data for these sensitive populations adequate for quantitative analyses?

TK data may be developed empirically or through compartmental or physiologically based TK models. It is recommended that these data, models, and approaches be evaluated for their appropriateness (IPCS, 2010; U.S. EPA, 2006a). For each critical effect identified for a particular agent, separate DDEF analyses are conducted for EF_{AK} and EF_{HK}. As such, data for multiple sensitive tissues/endpoints can be evaluated, concentrating on those effects that demonstrate response levels near the POD for the critical effect.

3.1.1. Dose Metric

Dose metric is a measure of the internal dose of a chemical agent. A dose metric associated with the health outcome of interest is most useful when it describes target tissue exposure in terms of the toxic chemical moiety (parent or metabolite) and is expressed in appropriate time-normalized terms. The choice of the dose metric is an important component in TK extrapolations. This choice depends on whether toxicity is best ascribed to a transient tissue exposure or a cumulative dose to the target tissue. For a given chemical, the appropriate dose metric will also be determined by, and can vary with, the MOA, duration of exposure, and the adverse effect of concern (U.S. EPA, 2006a). Selection of an appropriate dose metric based on specific endpoints involves several elements including:

DOSE METRIC

Dose metric is a measure of the tissue concentration of the toxicologically active chemical species that reflects a time-normalized (i.e., AUC) or instantaneous (i.e., C_{max}) measure of concentration. Dose metric values may also include measures of chemical flux or clearance. In some cases, dose metrics may be expressed in direct physiological units like glomerular filtration. (See the boron and compounds case study in Appendix A to this document.)

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

SELECTION OF THE APPROPRIATE DOSE METRIC

Dose metrics will differ with respect to the toxicological response of interest. While clearance values for some agents may be used to describe internal exposures, clearance may not be the dose metric most closely associated with the toxicological response of interest. Rather, the toxicological response may be mediated by the interaction of the toxicologically active chemical form with the receptors in the target tissue of interest, better represented by C_{max} or AUC values.

For example, the acute central nervous system effects of halogenated solvents may relate to C_{max} values for the parent compound in the brain, while chronically observed nephrotoxicity may best relate to averaged tissue concentrations of a metabolite or the rate at which metabolites are formed.

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

Clearance, while not often considered a dose metric, can be used in DDEF derivation. Clearance is mathematically inversely related to AUC (i.e., AUC = dose/clearance); thus, differences in clearance values can be used in the calculation of ratios. When metabolism represents the primary or sole clearance mechanism, either of two clearance models may be applicable. The first, intrinsic clearance (Cl_{int}), has been used for interspecies scaling of administered doses in drug development (Houston and Carlile, 1997) and is applicable at doses that do not result in metabolic saturation. Cl_{int} is calculated as a ratio of the theoretical maximal initial velocity of the reaction to the Michaelis constant (V_{max}/K_m) and is in units of volume of the substrate cleared per unit time, where K_m is the substrate concentration driving the reaction rate at one-half V_{max}. The Cl_{int} can be extrapolated to the whole body with knowledge of protein binding and the recovery of the protein or cellular or subcellular fraction used in the *in vitro* investigations (Carlile et al., 1997).

The second clearance model is hepatic clearance (Cl_{hep}), which is also based on V_{max}/K_m measurements but includes a substrate delivery term whose value is governed by hepatic blood flow. These measures of clearance differ in that Cl_{int} is not bounded by hepatic blood flow, but Cl_{hep} cannot exceed hepatic blood flow. While metabolic rate constants (V_{max} and K_m) derived from *in vitro* data can also be scaled up and incorporated into PBPK models, the use of the hepatic clearance model is a simpler approach when an appropriate PBPK model is unavailable. Classical, compartmental TK analyses and measures of clearance of the parent compound are best suited for conditions where metabolism represents a detoxication process, when substrate concentration is less than the K_m value, and when metabolism represents the major clearance mechanism.

INTRINSIC CLEARANCE

Cl_{int} is often calculated for therapeutics (as V_{max}/K_m). While it is a valuable measurement for purposes of comparing agents, it is not suitable for derivation of DDEFs. Measures of intrinsic clearance do not take into account the constraints of the intact system (e.g., partitioning into tissues, blood flow), which can limit metabolic clearance.

Whether toxicity is attributable to a parent chemical, a metabolite, or some combination of metabolites is a critical consideration. The active chemical moiety can be identified through studies in which the toxicities induced by the parent chemical and metabolite(s) are compared or from the results of studies using enzyme inhibitors and/or inducers. *In vitro* studies can also be quite useful in this regard under appropriate conditions (see Sections 2.4 and 3.1.3). Quantifying differences in dosimetry can be difficult when metabolic pathways become complex (e.g., where competition among pathways may be concentration dependent). If the metabolic pathway bifurcates and the identity of the bioactive metabolite(s) is unknown or unquantifiable, determination of the appropriate dose metric can be highly uncertain.

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

notwithstanding, measurements of internal dose in circulating blood (IPCS, 2005) may be used as the basis for DDEF derivation under either of the following conditions:

- When evaluating interspecies differences, the distribution from blood to target (critical) tissues is shown to be or can be assumed to be the same between animals and humans.
- When evaluating intraspecies differences, the distribution from blood to sensitive (critical) tissues is shown to be or can be assumed to be the same in segments of the human population representing those generally responsive and potentially sensitive human populations.

Confidence in model predictions is enhanced when predictions can be compared directly to observed data. However, few human data sets exist that describe concentrations of toxicants in solid tissues. Blood, however, is much more readily obtained, and so the ability to compare predictions of blood to observations is more readily accomplished. This situation results in a higher level of confidence in modeled blood concentrations compared to solid tissue concentrations. Since the partitioning of the active chemical from blood into systemic target tissues may be governed more by physicochemical properties than by biological processes, communication of the understanding of these processes will increase confidence in predictions of solid tissue concentrations. This difference in confidence in predictions may be considered another basis for relying on data describing the concentration and variability of the biologically active metabolite in the central compartment.[7] For example, the ratio of blood lipid to tissue lipid concentrations may be a key determinant in the diffusion of lipophilic compounds out of blood; however, differences in tissue lipid composition between species may be fairly small compared to differences in blood flow and metabolic activity.

3.1.2. Dose Selection

Because variability in internal dosimetry may be a function of dose, the selection of the external exposure (inhaled concentration or orally ingested dose) is important. In some cases, there may be nonlinearities between the external dose and the dose metric. That is, increasing or decreasing doses may not produce proportional increases or decreases in the dose metric. In this instance, the dose selected for the DDEF derivation will impact the magnitude of EF_{AK} or EF_{HK}. Using a dose at or near the POD alleviates some concerns regarding nonlinearities in

[7]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.

metabolism. This situation is especially true for interspecies extrapolation, where the basis for DDEF calculation is the dose metric. The human equivalent concentration (HEC) or human equivalent dose (HED) is defined as the human exposure producing the same level of the dose metric as attained in the animal at the POD. Alternatively, data that show a linear relationship between external dose and internal dose metrics will lessen this dependence of dose on the estimation of EF_{AK} or EF_{HK}; thus, doses that may be higher or lower than the POD for the critical effect can be used in the calculation.

3.1.3. *In Vitro* Data

Due to ethical and practical constraints, some studies with humans are not possible—especially with chemicals already known to be toxic. *In vitro* study designs offer excellent opportunities to assess the toxicity of an agent, especially when the need to isolate TK from TD is a concern. However, it is important when deriving EF_{AK} and EF_{HK} to consider interspecies differences in ADME. *In vitro* data can be used for quantitative purposes only when interpreted in the context of the intact system, as discussed in Section 2.4. Care must be taken to avoid taking isolated findings out of context.

***IN VITRO* CAVEATS**

When investigating toxicodynamic events, *in vitro* experiments offer the opportunity to control for TK influences; concentrations of the toxicant can be well controlled. Results of *in vitro* toxicokinetic/metabolism studies should be used only when interpreted in the context of the intact system. Samples used *in vitro* should closely represent the species/population of interest, and the measured response should be one well associated with the critical effect.

3.2. INTERSPECIES TOXICOKINETIC EXTRAPOLATION

This section provides a discussion of the quantitative differences in the TK between animals and humans that are used to compute EF_{AK}. In this process, TK differences between species are characterized as the ratio of applied (administered) doses in the test species and in humans (if human data or models are available) that result in the same level of the internal dose metric (see Figure 3). 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 and the applied dose are linear in the range of extrapolation. Predictions of dose metrics from verified TK models are often acceptable. Illustrative case studies are included in Appendix A.

3.2.1. Considerations for Interspecies Toxicokinetic Extrapolation Factor

TK differences between animals and humans are evaluated for the selected critical effect and for effects arising near the POD for the presumed 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. Selection of the dose metric is based on a weight-of-evidence approach emphasizing both qualitative and quantitative evidence. An important part of this process is evaluating concordance of metabolic processes between the animal model and humans. An additional consideration is whether the kinetic data are from a "typical" or average adult animal as opposed to an animal model system that may be unusually sensitive for a particular effect (e.g., metabolic knockout).

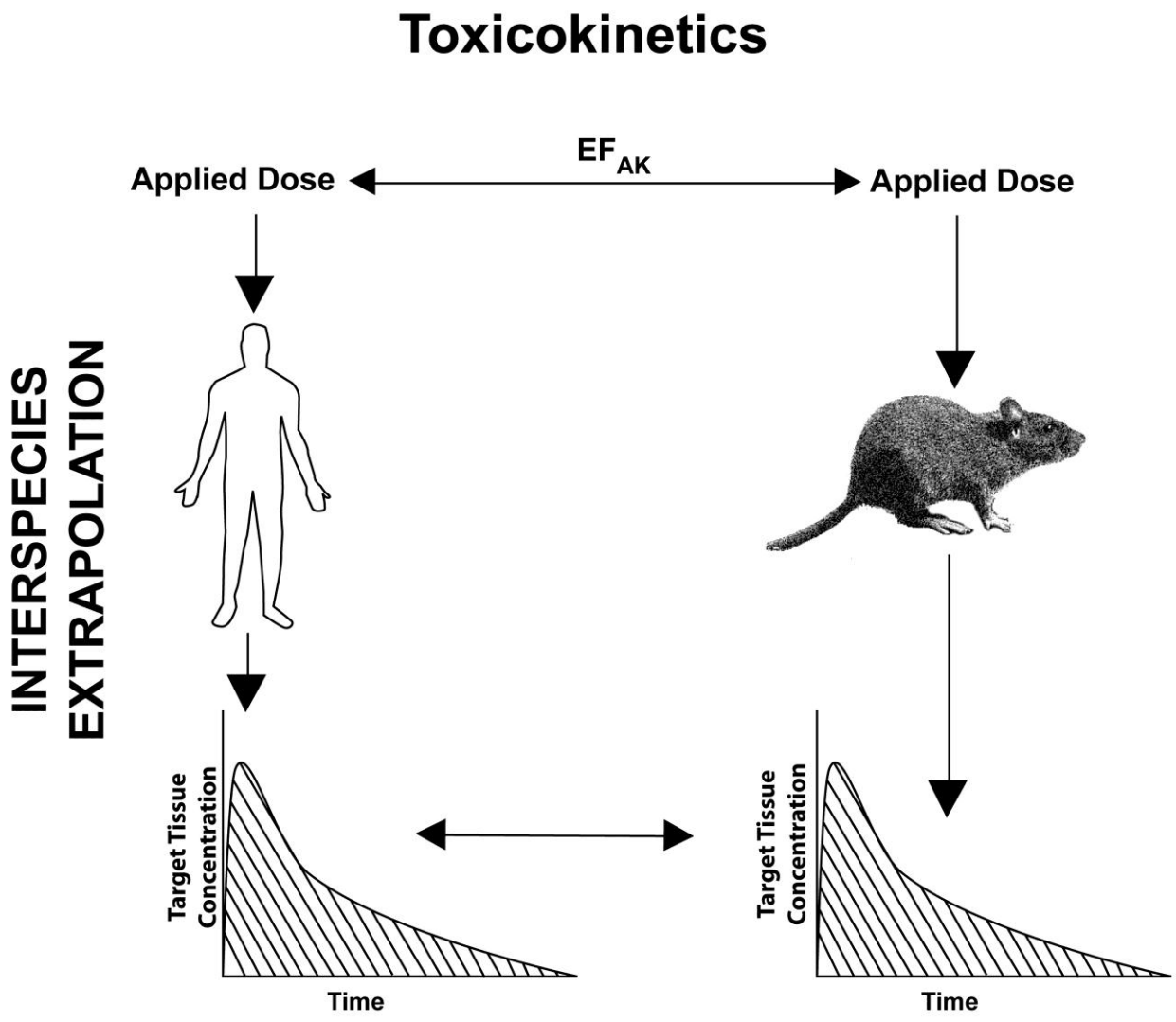

Figure 3. Interspecies toxicokinetics. In keeping with the principles established in earlier Agency guidance, which addressed inhaled toxicants (U.S. EPA, 1994), interspecies differences in TK are defined as differences in the external dose producing the same level of the dose metric in the target tissue of interest in test animals.

When animal data come from a group or from individuals expressing a condition known to be useful in identifying a sensitive human population or life stage, and when the corresponding population group in humans is determined to be the sensitive population, the extrapolation can be conducted between the sensitive animal and sensitive human. In this case, these data may cover both inter- and intraspecies extrapolation, and so represent both EF_{AK} and $EF_{HK,}$ respectively. However, it is recommended that a full weight-of-evidence evaluation be conducted. Using this example, if there are no data in the developing human, but data are available in the adult human, then the data-derived interspecies extrapolation would be from the more sensitive animal (e.g., sensitive life stage) to the general human population; intraspecies extrapolation would require default assumptions.

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

3.2.2. Computation

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

3.2.2.1. *Use of Toxicokinetic Models*

A PBPK (or other TK) model provides the most biologically appropriate approach for evaluating interspecies TK extrapolation. The model is subjected to evaluation as previously described (IPCS, 2010; U.S. EPA, 2006a). The model can be used in different ways, depending on the model and the circumstances. In some cases, the TK model may be used directly to perform interspecies extrapolation (i.e., to derive a human equivalent concentration or dose that includes TK considerations). The use of a PBPK model would obviate the need for EF_{AK}. In other cases, the TK model may be used to derive EF_{AK}.

3.2.2.2. *Use of Ratios*

When AUC or concentration at steady state is the relevant dose metric, and if animal and human data or TK models are available, EF_{AK} is derived using a ratio of external or applied

doses producing the same AUC value. This is accomplished by identifying doses associated with the AUC value produced in animals at or near the animal POD (AUC_A) (see eq 1 and Figure 4). The human dose that produces the same AUC_A value is the toxicokinetically equivalent dose. In these cases, differences between the animal and the human dose producing the same AUC value in each species define the EF_{AK}.

$$AUC = Dose \div Clearance \quad (1)$$

$$AUC_A = D_A \div Cl_A = D_H \div Cl_H$$

where,

D_A = animal external dose (administered or external dose to the test animal species that leads to a level of a toxicologically relevant dose metric at or near the POD)

Cl_A = animal clearance value

D_H = human external dose (administered or external dose to the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species)

ClH = human clearance value

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

$$EF_{AK} = \frac{D_A}{D_H} \text{ OR } \frac{Cl_A}{Cl_H} \quad (2)$$

where,

D_A = animal external dose (administered or external dose to the test animal species that leads to a level of a toxicologically relevant dose metric at or near the POD)

D_H = human external dose (administered or external dose to the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species)

ClA = animal clearance value

Cl_H = human clearance value

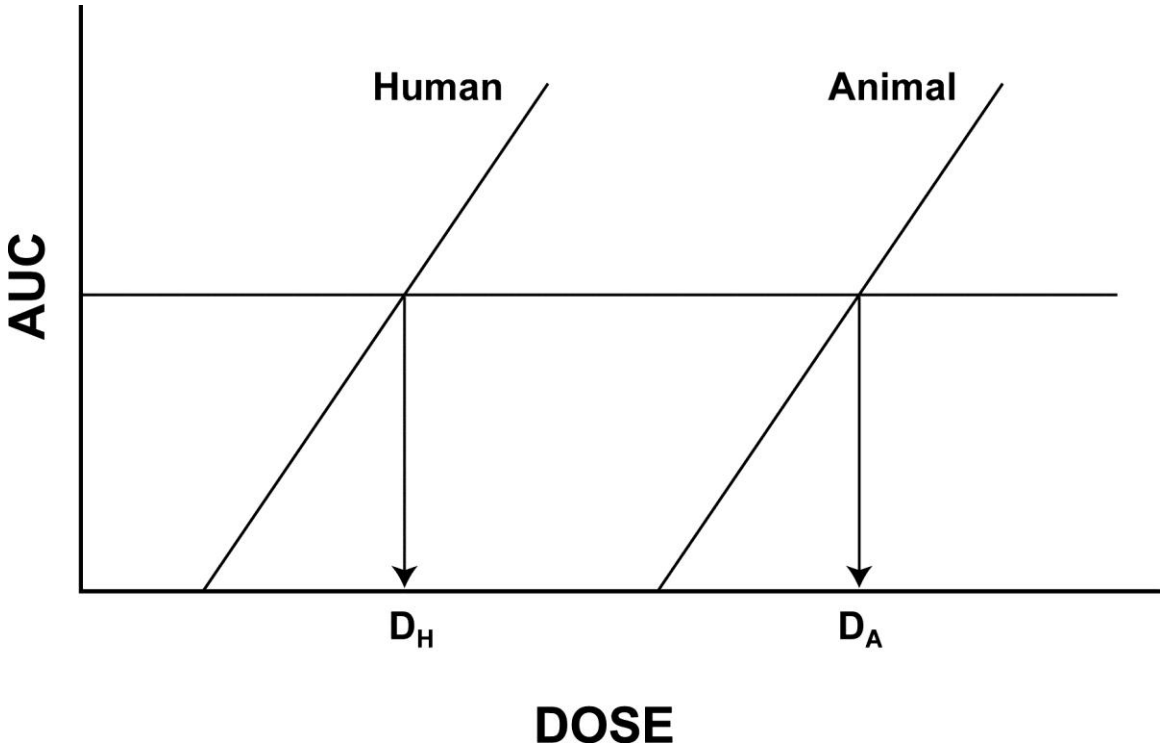

Figure 4. The conceptual relationship between dose and dose metric in animals and humans. Here, AUC is the appropriate dose metric, and the relationship between dose and AUC is determined in animals and in humans. This method can be used to develop a human equivalent dose or concentration, or in the calculation of EF_{AK}. Calculation of EF_{AK} requires knowledge of applied D_A and D_H that produce the AUC value determined in animals at the point of departure (i.e., AUC_A).

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

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

where,

D_A = animal external dose (administered or external dose to the test animal species that leads to a level of a toxicologically relevant dose metric at or near the POD)

AUC_A = area-under-the-curve value produced in animals at or near the animal POD

D_H = human external dose (administered or external dose to the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species)

This is graphically presented in Figure 4.

Because clearance values are the mathematical reciprocal of internal dose (i.e., AUC), they may also be used to calculate a DDEF value, with the human clearance value in the denominator. Calculations using C_{max} are developed in a manner similar to that for AUC. When the dose is lower in humans than animals at the same AUC or C_{max} value, the developed DDEF will be greater than 1, demonstrating that humans are more sensitive than animals.

3.2.3. Relationship to Other EPA Guidance

The development and use of data for model predictions for tissue dosimetry to serve as the basis for quantitative, interspecies extrapolation via DDEFs is consistent with existing EPA policy (U.S. EPA, 2006a, 2002b). EPA's inhalation RfC methodology presents a continuum of approaches from rudimentary knowledge to biologically based dose-response models (U.S. EPA, 2012, 1994). The RfC methodology describes default approaches for dosimetric adjustment of animal exposure concentrations based on categorical descriptions of target tissue and target tissue concentrations in test species and humans. The first is for reactive (Category 1) gases and inhaled particles that damage portal-of-entry (respiratory tract) tissues, and the second is for gases that are absorbed and produce toxicity in tissues bathed by circulating blood (Category 3 gases). For Category 1 gases, toxicity information identifies the affected region of the respiratory tract, and species differences in the regional respiratory tract surface area and airflow (respiratory rate) serve as the basis to quantify species differences in dosimetry. For Category 3 gases, species differences in the solubility of the compound in blood (the blood:air partition coefficient) serve as the basis upon which to quantify species differences in dosimetry. The default interspecies extrapolation approach for deriving an oral RfD is dose scaling by the ratio of species' body weights raised to the ¾ power $(\text{human BW:animal BW})^{3/4}$ (U.S. EPA, 2011). These guidance documents indicate that their approaches are default dosimetric adjustments, to be superseded when more detailed information on tissue dosimetry can be developed. The

subject of this DDEF guidance is the development and interpretation of quantitative TK data for the purpose of developing nondefault values for inter- and intraspecies uncertainty/extrapolation.

3.2.4. Conclusions for Interspecies Toxicokinetic Extrapolation Factor

Mathematically, EF_{AK} is the ratio of the external animal dose (at or near the POD) to the external human dose expected to result in the same level of the dose metric. This situation is mathematically analogous to developing the HEC or the HED.[8] If possible, EF_{AK} values should be calculated for multiple organs/effects. For a given organ or effect, the same level of the dose metric at the animal POD should be used for TK analyses conducted in test animals and humans. The quantitatively determined DDEF values for EF_{AK} will be less than 1 if the level of the dose metric at the animal POD is attained at a higher dose in humans than in animals (indicating that humans are less toxicokinetically sensitive).

EF_{AK} CAN BE LESS THAN 1

For EF_{AK}, central tendency estimates of doses or exposures producing the same measure of target tissue exposure in animals and humans are used. Lower sensitivity in humans compared to animals is demonstrated by lower AUC or C_{max} values, or higher clearance values in humans than in animals at the same exposure. In these instances, the calculated value of EF_{AK} will be less than 1.

Confidence in EF_{AK} is increased when decisions and calculations are well documented. This narrative includes descriptions of toxicity data identifying the target tissue, chemical species, MOA, and species concordance of effects. Data describing the TK, the metabolism of the compound, and the relationship between external dose and dose metric are also summarized. Data that show a linear relationship between external dose and internal dose metrics can be specifically reiterated in this description, which will indicate generalizability of the EF_{AK} value to doses that may be higher or lower than those used in DDEF calculation. Because animal-to-human differences in target tissue concentrations may not be consistent for all responding tissues or organs, a comparison of POD and DDEF values from multiple affected organs will increase confidence in the extent to which the developed DDEF value sufficiently addresses the toxic action of the assessed chemical. Results can be presented in tabular form for ease of comparison across endpoints.

3.3. INTRASPECIES TOXICOKINETIC EXTRAPOLATION FACTOR

When toxicity data defining the POD are developed in test animals, the established framework for UFs includes an initial extrapolation to the human population, then an

[8]If an HED or HEC value is developed, the residual one-half order of magnitude in the value for UF_A encompasses TD, as well as any residual uncertainty in the derived HEC or HED value.

extrapolation to account for human intraspecies or interindividual variation. The purpose of these analyses is to characterize the variation of TK among the human population, the quantitation of which will help avoid the application of default UFs. From a TK standpoint, among humans experiencing the same *external* dose, sensitivity is due to higher target tissue concentrations of the toxicant in the sensitive population or group relative to the rest of the general human population. As with interspecies extrapolation, the DDEF values may be compared to default UF values. This comparison is conducted to aid policy decisions and risk communication after the adequacy of the underlying data have been confirmed (a data quality evaluation). Characterization of the available data includes considering how completely the sensitive population has been identified and its sensitivity described (as opposed to assumed) (U.S. EPA, 2002b). The selection of a bimodal- or unimodal-based analysis will be a function of the available data. It is important to document the available information and related statistical analysis and/or assumptions that serve as the basis for selecting a unimodal or a bimodal distribution of sensitivity. The extent to which this description has been done will inform decisions regarding the application of DDEF analyses.

Sensitivity may reside in an identifiable population (e.g., distinct life stage or genetic polymorphism) or may be less distinctly distributed among humans (e.g., differences in the levels of an endogenously expressed enzyme). As described in more detail below, extrapolation among the human species is accomplished by either of two options (or both): (1) evaluating human interindividual variability among the entire human population or (2) explicit identification of the potentially sensitive population(s) for TK analysis. Differences in the scope/intent of the risk assessment and the availability of data to identify a given population or group as sensitive (e.g., the aged, those with genetic polymorphisms) may limit application of the second option. This section provides a discussion of the quantitative differences in TK among humans for intraspecies extrapolation (i.e., EF_{HK}).

Although it is important to acknowledge the complex factors that contribute to human variability, for sake of simplicity, the entire human population can be distinguished as those who are sensitive and others that make up the general population. This is an important distinction, influencing the choice of computational methods. A bimodal analysis (segregating the entire population into the general and the sensitive populations) is used when sensitive individuals can be identified on the basis of physiological, biochemical, or life-stage attributes and grouped into a distinct population. A unimodal analysis is used when sensitive individuals cannot be identified *a priori* on the basis of physiological, biochemical, or life-stage attributes. Regardless of the analysis type, it should be based on a sufficiently large and diverse population data set, including adequate sampling of potentially sensitive populations and life stages.

3.3.1. Considerations for Intraspecies Toxicokinetic Extrapolation Factor

3.3.1.1. *Sensitive Populations*

This section presents the process of considering the TK of an agent among the human population. For some chemicals, data may be sufficient to identify one or more sensitive populations but insufficient for other chemicals. Sensitivity in the human population may be due to life stage, health status or disease state, genetic disposition, and other factors (also see Section 2.1.2). Critical windows of development, and therefore "windows of susceptibility," occur at different times for various tissues, organs, and systems; therefore, considering sensitivity to more than one critical effect may require consideration of more than one life stage. As discussed in more detail below, distributional analysis of response data can be conducted to identify points for use in quantitation. In completing the analysis, it is important to describe the relationship between the dose metric and the toxicity endpoint(s) of concern (e.g., critical effect or key event). The intraspecies extrapolation step is intended to account for differences between the central tendency of the entire population and the sensitive portion of the population (unimodal analysis) or between the central tendency of the general population and some point in the distribution of the population of sensitive individuals (see bimodal analysis; Section 3.3). Considering sensitivity to more than one critical effect may require consideration of more than one potentially sensitive population.

From a TK standpoint, among humans experiencing the same *external* dose, sensitivity is due to higher target tissue concentrations of the toxicant in the sensitive population relative to the rest of the human population. Higher tissue concentrations can be demonstrated as higher AUC or C_{max} values, or by lower Cl values. Thus, when assessing sensitivity, values in the upper tail of the distribution (e.g., 95th, 97.5th, 99th percentiles) of values for AUC and C_{max} values are considered, while values in the lower tail of the distribution (e.g., 1st, 2.5th, 5th) of values for Cl are considered. The examples demonstrated in this section use AUC or C_{max} as the dose metric. Equation 4, discussed in Section 3.3.2.2, demonstrates the mathematical approach to quantitation of EF_{HK} based on AUC, C_{max}, or Cl. For AUC and C_{max} values, EF_{HK} is computed as the ratio of an internal dose metric attained in the sensitive population to that observed at or near the central tendency in the general human population exposed to the same external dose or concentration. Illustrative examples are included in Appendix A.

Sensitivity may be due to increased tissue exposure at a given dose (TK) or to increased responsiveness to a given tissue concentration (TD; see also Section 4.3.1.1). With an adequate description of the population variability of biochemical, physiological, and anatomical variability, a distributional analysis of the dose metric can confirm that TK variability influences sensitivity. If the dose metric is segregated into distinct groups and the supposed sensitive population has a higher level of the dose metric (e.g., AUC or C_{max}) than the rest of the

population (see Figure 5, left panel), these results would confirm that sensitivity may be at least partially influenced by TK. However, if the dose metric demonstrates a uniform distribution (see Figure 5, right panel) and sensitive individuals are distributed among the entire population distribution, such results would suggest that TK variability may have little influence on sensitivity. Ideally, data will be complete enough to enable more than point estimates among the populations. As discussed in more detail below, distributional analysis of response data should be conducted to identify points for use in quantitation. In completing the analysis, it is important to describe the relationship between the dose metric and the toxicity endpoint of concern (e.g., critical effect or key event). A sufficiently large and diverse population data set must be used to ensure that it includes an adequate sampling of potentially sensitive populations and life stages.

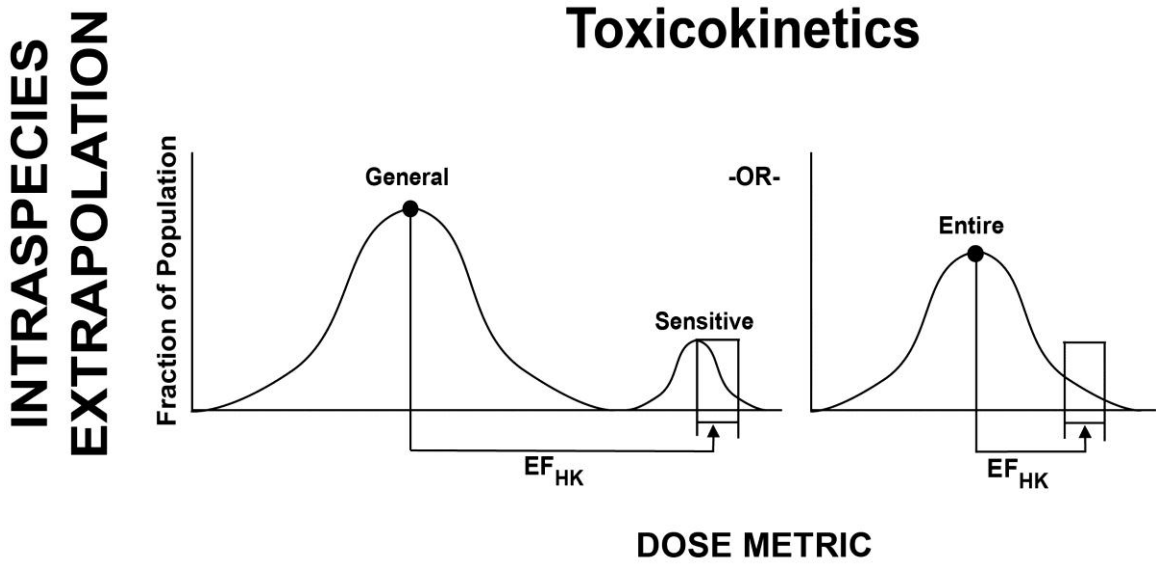

Figure 5. Intraspecies toxicokinetics. Dose metric values may be distributed among the human population in a bi- (or multi-) modal or a unimodal fashion. Even when an identifiable population is presumed or demonstrated to be sensitive on the basis of exposure, the distribution of dose metric values may be unimodal. Differences in distribution type affect quantitative methods as described in the text. EF_{HK} is computed on the basis of differences in dose metric attained at the same external dose. This figure demonstrates analysis of AUC and C_{max} data.

3.3.1.2. *Target Tissues*

When responses are observed in several organs at or near the POD for the most sensitive effect, a comparison of EF_{HK} values developed for those tissues is informative. However, the selection of a target organ for calculating human variability other than the one serving as the

basis for animal-to-human extrapolation is best accompanied with a justification. Development of candidate DDEF values should be undertaken in a manner analogous to the development of candidate RfD or RfC values per EPA (U.S. EPA, 2002b). The extrapolation approach is generally most consistent when the same tissue or organ is used for each phase of the extrapolation procedure.

3.3.1.3. *Dose-Response*

Because variability in internal dosimetry may be a function of dose, the selection of the external exposure (e.g., inhaled concentration or orally ingested dose) is important for use in estimating intraspecies differences in dosimetry. When the POD is derived in animals, several options exist for interspecies extrapolation, depending on the availability of suitable animal and human data describing TK and/or TD. In the TK part of the interspecies extrapolation step, the animal POD is extrapolated to produce a toxicokinetically equivalent human dose or concentration. In some instances, interspecies extrapolation may also include a separate, technical treatment of TD. Also, interspecies adjustment may be completed by the default value for UF_A, or the POD may be defined from dose-response studies in humans. Regardless of whether the default UF is applied or DDEF value is computed to complete *interspecies extrapolation*, it is the external dose (mg/kg-day) or concentration (mg/m^3) reflecting this adjustment that is most appropriate for application in determining human *intraspecies* TK variability.

The basis for comparison of *human variability* is at the level of the internal dose metric rather than the external dose. This choice for the level of comparison is consistent with the principle that it is the target tissue dose that drives the toxic response, not an external (applied) dose. Placing the comparison at the level of the target tissue dose (dose metric, internal dose) forces the assumption of TK linearity to maintain consistency with the established approach of applying UFs to external doses. Thus, it is important that the relationship between internal and external doses be well characterized to ensure TK linearity (the proportionality of the ratio of external dose to dose metric across some range of doses or exposures). While nonlinearities may become evident at substantially different human exposures (e.g., between the interspecies-adjusted POD and the resulting RfD or RfC), the prime point for comparison is the range of doses (or concentrations) immediately surrounding the interspecies-adjusted POD. Because EF_{HK} and preceding DDEF values will be used to extrapolate external doses, it is recommended that TK linearity be tested by documenting a consistent ratio of external doses and dose metric values in a range of exposures bounded by the value of EF_{HK}.

Nonlinearities in TK frequently arise due to metabolism, and are addressed on a case-by-case basis. Several circumstances may account for nonlinearities. When a chemical is

metabolized by a single enzyme and the concentration is below the K_m, the metabolic rate is essentially defined by V_{max}/K_m—metabolism is essentially first order. When concentrations become saturating, further increases in concentration do not result in increased metabolism. When metabolism represents a bioactivation process, no further increase in toxicity due to a bioactivated metabolite is anticipated. However, if metabolism represents a detoxication process, then a disproportionate increase in toxicity from the accumulation of the toxic parent chemical may be predicted. However, increasing concentrations of the chemical may recruit additional enzymes with a lower affinity, and these enzymes may be responsible for the production of detoxicated or bioactivated metabolites. Nonlinearities represent special cases, and when identified, are seldom evident over narrow ranges of concentrations. This DDEF guidance is concerned with the factors governing inter- and intraspecies extrapolation. When nonlinearities become evident in the range of doses pertinent to these dose extrapolation steps, they are considered on a case-by-case basis. Consideration of nonlinearities that are evident across broader ranges of concentrations (e.g., animal POD versus RfD values) are beyond the scope of this guidance.

Specific differences among humans, particularly those demonstrated *in vitro* (i.e., intrinsic clearance), are most reliably used when they are translated into differences in dosimetry based on the anatomical and physiological constraints imposed by the intact system (whole animals). This approach may include evaluations of multiple different doses.

3.3.2. Computation

For intraspecies TK extrapolation, differences in dosimetry are characterized for the human population by comparison of central tendency TK data for the sensitive population to measures of the general population or the entire population (see Section 3.3.1.1, Figure 5). The comparisons are among differences in internal dosimetry (or target site dose, dose metric) resulting from the same external exposure. To address human variability, some attention is devoted to documenting the reasons for the assumption of sensitivity among any population anticipated *a priori* to be sensitive (generating a bimodal analysis, which involves the general and sensitive populations). In the absence of a specifically identifiable population as sensitive, or when the analysis of TK among humans includes a separate population presumed to be sensitive fails to result in distinguishable distributions of the dose metric, a unimodal type analysis should be conducted. In this instance, comparisons of the dose metric between the central tendency and defined percentiles of the entire population distribution (upper percentiles for C_{max} and AUC; lower percentiles for clearance) can be made. Regardless of the distribution type, the presentation of multiple values/points in the tail of the distribution will better enable risk communication and management decisions.

3.3.2.1. *Use of Toxicokinetic Models*

A PBPK or other TK model provides the most biologically appropriate approach for evaluating intraspecies TK extrapolation. When a model is available and has been properly evaluated (IPCS, 2010; U.S. EPA, 2006a). It can be used in different ways depending on the model and the circumstances. There are several potential applications of TK models for sensitivity among humans. Some examples include the identification of the POD in a test animal species and a subsequent two-step process to perform a TK extrapolation to account for inter- and intraspecies differences. This case is the typical scenario, and in it, the initial extrapolation is from the animal POD value to a value representing the central tendency in the human population. If TK data or a reliable TK model exists that can be used to examine dosimetry in the segment of the human population deemed sensitive, then the second extrapolation step to account for human variability is undertaken to define differences in tissue concentrations between the central tendency of the general population and those in the sensitive population or in the sensitive portion of the population. For example, the DDEF value for EF_{HK} is determined as the ratio $Concentration_{sens}$:$Concentration_{gen}$.

A second circumstance is exemplified when the POD is identified in the generally responsive (e.g., 70-kg adult human male), obviating the need for interspecies extrapolation and requiring an extrapolation to account for sensitivity among humans. In this case, the data or the model used to identify the dose metric near the central tendency of the general (overall) human population and in the segment of the population representing sensitivity/susceptibility will be examined. However, when the POD is determined in the portion of the human population that is sensitive, deriving UFs or DDEF values for inter- or intraspecies extrapolation is unnecessary.

3.3.2.2. *Use of Ratios*

In addition to predictive models, EF_{HK} can be derived using a ratio (see eq 4). The value for the dose metric employed as the central-tendency measure in humans (e.g., area under the concentration-time curve at a fixed external dose at a measure of central tendency in the entire or general human population [AUC_{gen}]) would be that level of the dose metric identified from studies with animals and further extrapolated to account for interspecies TD differences, or from studies in humans. The value for the dose metric in sensitive populations or at a percentile of the entire population (e.g., area under the concentration-time curve at a fixed external dose at a percentile of interest for the sensitive human population or representing sensitive individuals among the entire human population [AUC_{sens}]) should be determined from empirical data or pharmacokinetic modeling.

$$EF_{HK} = \frac{AUC_{sens}}{AUC_{gen}} \text{ OR } \frac{C_{max\ sens}}{C_{max\ gen}} \text{ OR } \frac{Cl_{gen}}{Cl_{sens}} \quad (4)$$

where,

EF_{HK} = intraspecies TK extrapolation factor

AUC_{gen} = general human population AUC value (area under the concentration-time curve at a fixed external dose at a measure of central tendency in the entire or general human population)

AUC_{sens} = sensitive human population AUC value (area under the concentration-time curve at a fixed external dose at a percentile of interest for the sensitive population or representing sensitive individuals in the entire human population)

$C_{max\ gen}$ = general human population maximum concentration value (at a fixed external dose at the central tendency in the entire or the general human population)

$C_{max\ sens}$ = sensitive human population maximum concentration value (at a fixed external dose at a percentile of interest for the sensitive population or representing sensitive individuals in the entire human population)

Cl_{gen} = general human population clearance value (at a measure of central tendency in the entire or general human population)

Cl_{sens} = sensitive human population clearance value (at a percentile of interest in the sensitive human population or representing sensitive individuals among the entire human population)

3.3.3. Conclusions for Intraspecies Toxicokinetic Extrapolation Factor

EF_{HK} is a comparison of dose metrics resulting from the same external dose across the human population(s). When using an empirical ratio, EF_{HK} is the ratio of the dose metric value at a percentile of the distribution intended to represent the sensitive population or individuals and the dose metric value at a central-tendency measure of the general or the entire population. By quantitatively, EF_{HK} cannot be less than 1.

The dose selected for quantifying human interindividual variance may have an impact on the magnitude of variability (the DDEF value). When the POD is identified in a test species, the dose adjusted from the animal POD to account for all components of UF_A is the preferred dose for quantitation of human variability. If the POD is identified in humans not deemed to represent a human population, then human variability should be characterized at that dose. DDEF values should be calculated for multiple organs/effects when multiple tissues respond near the POD for

the most sensitive tissue (the critical effect). The consistency of DDEF values should be evaluated over a range of doses surrounding the POD to increase the level of confidence. DDEF values should be developed for each of the candidate tissues or effects, combined with POD values for each candidate tissue or effect, and clearly communicated. To ensure transparency, associated documentation should describe the mathematical method employed, the type of distribution and percentile(s) of interest, the rationale for choosing percentiles of interest, the dose metrics (e.g., AUC of parent compound in kidney), and the target tissues for which EF_{HK} values are developed. Confidence in the extrapolation is improved when distribution types are justified or explained.

4. DATA-DERIVED EXTRAPOLATION FACTORS BASED ON TOXICODYNAMICS

4.1. GENERAL CONSIDERATIONS

TD describes the critical interaction of the toxicologically active chemical moiety with the target site and the ensuing sequence of events leading to toxicity. Data that describe the dose-response relationship serve as the basis for deriving extrapolation factors for TD components. This section provides a discussion of factors common to inter- and intraspecies extrapolation for TD. In contrast to TK, which focuses on *differences in internal dosimetry,* TD differences are quantified as *differences in concentration*[9] *producing the same level of response* between test animals and humans, as well as among humans. TD evaluations may include multiple response levels, critical effects, key events, or analytical methods. Developing a DDEF for TD requires knowledge about the relationship between an event measured *in vitro* or *in vivo* (e.g., receptor binding) and the end result (critical effect). Doses or concentrations producing the measured event can be obtained *in vitro* or *in vivo* in the tissue of interest or a suitable surrogate. TD models, the most complex of which may be represented by BBDR models, are an important tool through which predictions may be made to extend the range of empirical observations. Comparisons between animals and humans or to quantify human interindividual differences are made on the basis of doses or concentrations that produce the same level of the same measured response.

Quantitative differences between animals and humans are used for EF_{AD}, whereas differences in sensitivity within the human population are used for EF_{HD}. Thus, EF_{AD} is used to extrapolate findings in laboratory animals to the general human population, and EF_{HD} is used to extrapolate to sensitive human populations. The TD components (EF_{AD}, EF_{HD}) are combined with the corresponding TK components to develop the composite factor.

In rare cases, when a sensitive population or life stage has been identified, TD data in animals and the sensitive population (if available) may be employed to develop a DDEF value that combines both inter- and intraspecies TD extrapolation. In this case, the DDEF value derived represents both EF_{AD} and EF_{HD}. Because this is expected to be a rare situation, this section will address extrapolations separately.

Although a complete mechanistic understanding is not required, derivation of a DDEF for TD relies on some understanding of an MOA for the critical effect(s) identified for risk

[9]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.

assessment. As for all DDEF derivations, adequate and appropriate data are essential. Important issues to address include the following:

- What are the critical effect(s) and POD(s) being used in this assessment? If more than one, each should be considered in this process.
- What is the MOA, AOP, or mechanism(s) for that toxicity? Have the key events been identified? How are they measured?
- Is the MOA, AOP, or mechanism(s) in the animal model relevant to humans (qualitatively and/or quantitatively)?
- For interspecies extrapolation, are there sufficient data on the key events amenable to modeling such that a uniform measure of response 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?
- For intraspecies extrapolation, are there adequate data in human populations that describe population variation in response as a function of internal dose metric? Do the available data identify sensitive population(s)? Can the degree of this sensitivity be adequately estimated?

4.1.1. Mode of Action

TD extrapolation should be endpoint driven (considered in the context of the critical effect). For clarity, the choice of critical effect is justified, when possible, based on findings of response in exposed humans (U.S. EPA, 1994). Understanding MOA for the agent(s) of interest ensures that the TD responses (the biological events) used to derive the DDEF are relevant to the adverse health outcome of interest. These responses could include receptor affinity, enzyme inhibition, and molecular changes, among others. Repair of deoxyribonucleic acid (DNA) or tissue damage, biological thresholds, residual function, and other processes that could contribute to the shape of the dose-response curve and effects are considered. Experimental systems and measured responses in each species should be the same or comparable for both inter- and intraspecies extrapolation.

4.1.2. Relating Response to Dose or Concentration

TD data may be developed from the results of *in vivo* or *in vitro* studies. In some cases, *in vivo* data may be used, but care should be taken to control for the influence of TK. If there are existing human data measuring the response, then those data can be used to derive the POD,

thereby removing the need for the UF_A. Likewise, data from the most sensitive human population, if available, could be used for the risk assessment and a UF_H may not be needed.

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

When using *in vitro* systems, the response measured should be representative of the toxicity; that is, the measured endpoint should be consistent with or comparable to the critical effect or key event observed *in vivo* (if known). In cases where the measured response *in vitro* is known to differ from that observed *in vivo*, it is important to describe the potential impact of these differences on the final DDEF. For the sake of transparency when using *in vitro* data, the comparability of chemical uptake between animal and human tissues or preparations is addressed. Among the factors considered when presenting *in vitro* results are data describing metabolism of the test chemical by the *in vitro* system used.

4.1.3. Range of Doses or Concentrations

The relationship between the doses, tissue concentrations, and/or *in vitro* concentrations used to derive the DDEF, and those attained in the toxicity studies from which the POD is derived, can be characterized to improve transparency of the assessment. Optimally, the concentrations used in studies of the critical effect(s) include the concentration at the POD. The need for quantitative data is important because the variability in the response may change with increasing or decreasing dose or concentration. Doses and/or tissue concentrations may also be compared to those expected from environmental exposure; this comparison is part of the consideration of overall relevance of the test system.

4.2. INTERSPECIES TOXICODYNAMIC EXTRAPOLATION FACTOR

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

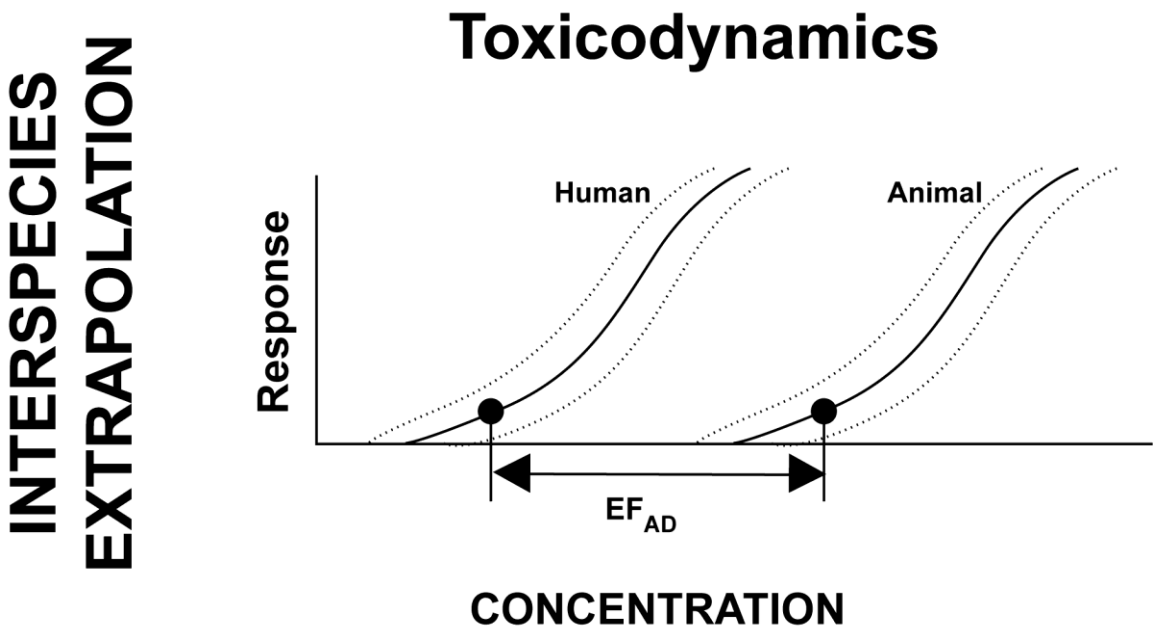

Figure 6. Interspecies toxicodynamics. The TD difference between test animals and humans is calculated from concentration-response relationships determined in test systems or animals generally representative of the respective species. The comparison is made for concentrations producing the same level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

4.2.1. Considerations for Interspecies Toxicodynamic Extrapolation Factor

4.2.1.1. *Mode of Action*

Endpoint(s) must be chosen for which to derive the EF_{AD}, and the endpoint(s) should be the actual critical effect or a key event in the MOA or AOP. For each endpoint evaluated, it is determined whether the data are from an animal typical of the responding species/strain/sex/life stage, as opposed to an animal model system that may be unusually sensitive for a particular effect. Characterization of the test animal might be important in transgenic animals and in animal models used to study specific human diseases (e.g., spontaneously hypertensive rats). Animal models and the MOA(s) based on them are best evaluated for human relevance (Meek et al., 2003), as has been done for forestomach tumors (Proctor et al., 2007).

4.2.1.2. *Target Tissues*

Data from the target tissues are preferred but not always available for human tissue. 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 provided there is sufficient justification 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 effect on this target enzyme in blood correlates very well and is entirely predictive of the effect in the target organ, and that the enzyme structure, function, and chemical affinity are the same regardless of tissue. Such information would be necessary to justify 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 several factors:

- Completeness of dose-response data,
- Shape of the dose-response curves, and
- Understanding of the effect along the toxicity pathway at that response level.

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

4.2.1.4. *In vitro Data*

When using data from *in vitro* systems, the activity of the parent chemical and/or metabolites, as well as the extent of metabolism of the compound by the *in vitro* system, should be known. The *in vitro* assay should measure a response that can be linked to the toxic outcome, and the assays should employ the same (responding) tissues from the *in vivo* test animal species/strain/sex/life stage and from humans. Experimental systems should be as closely matched as possible, and the concentrations of toxicant *in vitro* should be compared to tissue concentrations (the dose metric) at the POD. See Section 2.4 for other general considerations.

4.2.2. Computation

4.2.2.1. *Use of Ratios*

When TD models are not available, EF_{AD} can be calculated as a ratio describing the relationship between the tissue concentrations producing a set response in human tissues compared to animal tissues, preferably at or near the response level at the POD. For example, "*x* response level" may be a 10% response if the effective dose producing a 10% response rate or lower confidence bound on benchmark dose corresponding to a 10% increase in response ($BMDL_{10}$ value) is used. The EF_{AD} value would be calculated as follows:

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

where,

EF_{AD} = factor for interspecies extrapolation covering TD

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

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

To evaluate the extent to which the shape of the dose-response curve varies between animals and humans, and thus impacts the magnitude of EF_{AD}, a range of response levels should be evaluated (e.g., in cases where the dose-response data are extrapolated to derive levels for the POD). The rationale and implications for choosing the point for extrapolation should also be presented.

4.2.2.2. *Biologically Based Dose-Response Models*

A BBDR model often provides the most robust approach for evaluating interspecies extrapolation. These models are typically expansions of PBPK models (addressing TK) extended to include TD. By including both TK and TD components, BBDR models provide a linkage between external (applied) dose and biological response. Such models incorporate data from key events allowing direct estimation of adverse health outcome. In cases where the ultimate biological effect modeled in the BBDR model (e.g., DNA binding of the toxicant) is not the adverse health outcome of interest (e.g., tumor development), additional considerations are

needed. Perhaps the most important among these is the quantitative relationship between the modeled biological effect and the adverse health outcome. In keeping with principles established herein and elsewhere in Agency guidance, several points are important when evaluating a BBDR model for quantitative reliance. Fundamentally, the TK components of the model must be evaluated according to established principles (U.S. EPA, 2006a). TD components of the model should be evaluated according to principles established in this section. Because BBRD models translate exposure to response (without necessarily separating out TK from TD influences), they may be used to complete species extrapolation without developing distinct values for EF_{AK} and EF_{AD}. Alternatively, this human exposure may be identified as the HEC (for inhaled toxicants) or an HED (for orally encountered toxicants).

4.2.3. Conclusions for Interspecies Toxicodynamic Extrapolation Factor

Mathematically, EF_{AD} will be the ratio of the concentrations or dose metric values resulting in the same level of response in both the test species and human. DDEF values may be calculated for multiple PODs and organs/effects, but the response levels used for quantitation should be the same in animals and humans. Confidence in the value (knowing whether the DDEF value can vary depending on the response level) is improved when EF_{AD} values developed from multiple points on the concentration-response curve are comparable; the shape/slope of the curves may also influence these values. Quantitatively, EF_{AD} can be less than 1 if the data show humans are less sensitive than test species.

Preferably a summary of all conclusions and their scientific support are provided. Data describing the dose-response of the compound in animals and humans can be specifically reiterated in the summary. Finally, the summary also indicates the extent to which the EF_{AD} value can be generalized to doses that may be higher or lower than those used in its calculation.

4.3. INTRASPECIES TOXICODYNAMIC EXTRAPOLATION FACTOR

This section provides information for calculating TD variability within the human population. EF_{HD} is calculated as the ratio of concentrations or dose metric values producing the same level of the response at or near the central tendency in the general (nonsensitive) or the entire population to concentrations of the dose metric observed/predicted in the sensitive population or sensitive portion of the entire. From a TD standpoint, sensitivity 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 sensitive populations may be considered.

4.3.1. Considerations for Intraspecies Toxicodynamic Extrapolation Factor

4.3.1.1. *Sensitive Populations*

Sensitivity in the human population may be due to life stage, health status or disease state, genetic disposition, or other factors as discussed in Section 2.1.2. Critical windows of development, and therefore windows of sensitivity, occur at different times for various tissues, organs, and systems. Considering sensitivity to more than one critical effect may require consideration of more than one life stage. Data to address differential sensitivities in dynamic response are becoming more available with advances in the science (IOM, 2013). For example, population variation, such as genetic polymorphisms, is an expanding area of study. The increased availability and application of newer technologies, such as omics and high through-put, will benefit the derivation of DDEFs in general, and EF_{HD} in particular. A data-derived EF_{HD} is feasible if human data are of sufficient quality, the data address aspects of the critical effect consistent with that identified from applicable human or animal studies, studies have been conducted in the segment(s) of the population deemed sensitive, and/or a sufficiently large and diverse sample set is available.

For quality purposes, data should be sufficient to enable at least a rudimentary understanding of the distribution of values. As presented in Section 3.3.1.1, sensitivity may be distributed among the entire population or segregated into an identifiable sensitive population, dictating a unimodal or bimodal distribution, respectively, with their inherent DDEF quantitative procedures. As discussed in more detail below, distributional analysis of response data may be conducted to identify points for use in DDEF quantitation. The relationship between the measured response and the toxicity endpoint of concern (e.g., critical effect or key event) should be described, whether determined *in vivo* or *in vitro*.

4.3.1.2. *Target Tissues*

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

4.3.1.3. *In vitro Data*

Given the constraints on generation of human response data *in vivo*, *in vitro* studies offer an alternative. Samples selected for *in vitro* investigation should represent the central tendency of the human population as well as sensitive populations or life stages (see Section 2.4 for other general considerations). It is important that the *in vitro* assay measure a response that can be linked to the toxic outcome, and also that the assays employ the same (responding) tissues or

suitable surrogates for the tissues identified in the *in vivo* test animal species/strain/sex/life stage and from humans. Finally, experimental systems for each segment of the human population should be as similar as possible, and the concentrations of the toxicant *in vitro* be compared to tissue concentrations (the dose metric) at the POD.

4.3.2. Computation

For intraspecies TD extrapolation, differences in response are characterized for the human population by comparison of the TD data (doses or concentrations producing the same level of response) for the central tendency of the populations to the sensitive population or segment of the population. For quantitation, data on the critical response(s) are derived from a population that includes sensitive populations. Because the data available to define potentially sensitive populations from which to derive quantitative differences could be viewed in different ways, a statistical analysis may be helpful to determine distribution type (see Figure 7), which include:

- A unimodal distribution where the sensitive population represents the tail of the distribution because the sensitive individuals cannot be separated from the remainder of the human population; a sufficiently large and diverse population data set must be used to ensure that it includes an adequate sampling of potentially sensitive populations and life stages.
- A bimodal (or multimodal) distribution where the sensitive populations have been identified or suggested.

4.3.2.1. *Use of TD Models*

In the absence of *in vivo* response data in the sensitive human population, a BBDR or other TD model provides the best approach for evaluating intraspecies TD extrapolation. When sufficient data are available, these TD models can be structured and used to include differences in MOA components that may be life-stage dependent or influenced by other conditions potentially conferring increased sensitivity, such as genetic polymorphisms. Specific to EF_{HD}, it is critical that the model parameter(s) conferring increased sensitivity be well documented. When an appropriate model is available, it can be used in different ways depending on the model. In some cases, the TD model may directly account for interindividual variation and/or include data from the sensitive population, thus eliminating the need for EF_{HD}. In other cases, the TD model may be used to derive EF_{HD}.

4.3.2.2. *Use of Ratios*

When TD models are not available and populations can be identified as sensitive, then the ratio approach may be used. EF_{HD} is defined as the ratio between the concentrations producing the same level of response at the central tendency of the general population and a specified percentile of the distribution in the sensitive populations and/or life stages. EF_{HD} is calculated using eq 6 and described in Figure 7.

$$EF_{HD} = \frac{Concentration_{gen}}{Concentration_{sens}} \quad (6)$$

where,

EF_{HD} = factor for intraspecies extrapolation covering TD

$Concentration_{gen}$ = concentration producing the response corresponding to the POD at a measure of central tendency in the entire or the general human population

$Concentration_{sens}$ = concentration producing the response corresponding to the POD at a percentile of interest for the sensitive human population

When sensitivity among the population exhibits a unimodal distribution, EF_{HD} is the ratio of the concentration that elicits a level of response at the central tendency of the entire distribution to the concentration that elicits the same level of response at a lower sensitive percentile of the distribution. It is important to define and justify the point(s) in the distribution representing sensitivity; a sufficiently large and diverse population data set must be used to ensure that it includes an adequate sampling of potentially sensitive populations and life stages.

When sensitivity among the population exhibits a bimodal (or multimodal) distribution, the DDEF is determined in a similar manner, using the concentrations that elicit the specified level of response at the central tendency of the general population and in the lower tail of the sensitive population. The values selected to describe the sensitive population are defined and presented. The selection of the response level and the percentile of the distribution used to describe the potentially sensitive populations is an important issue. A justification for selection of the response level and percentile for sensitive population should be provided.

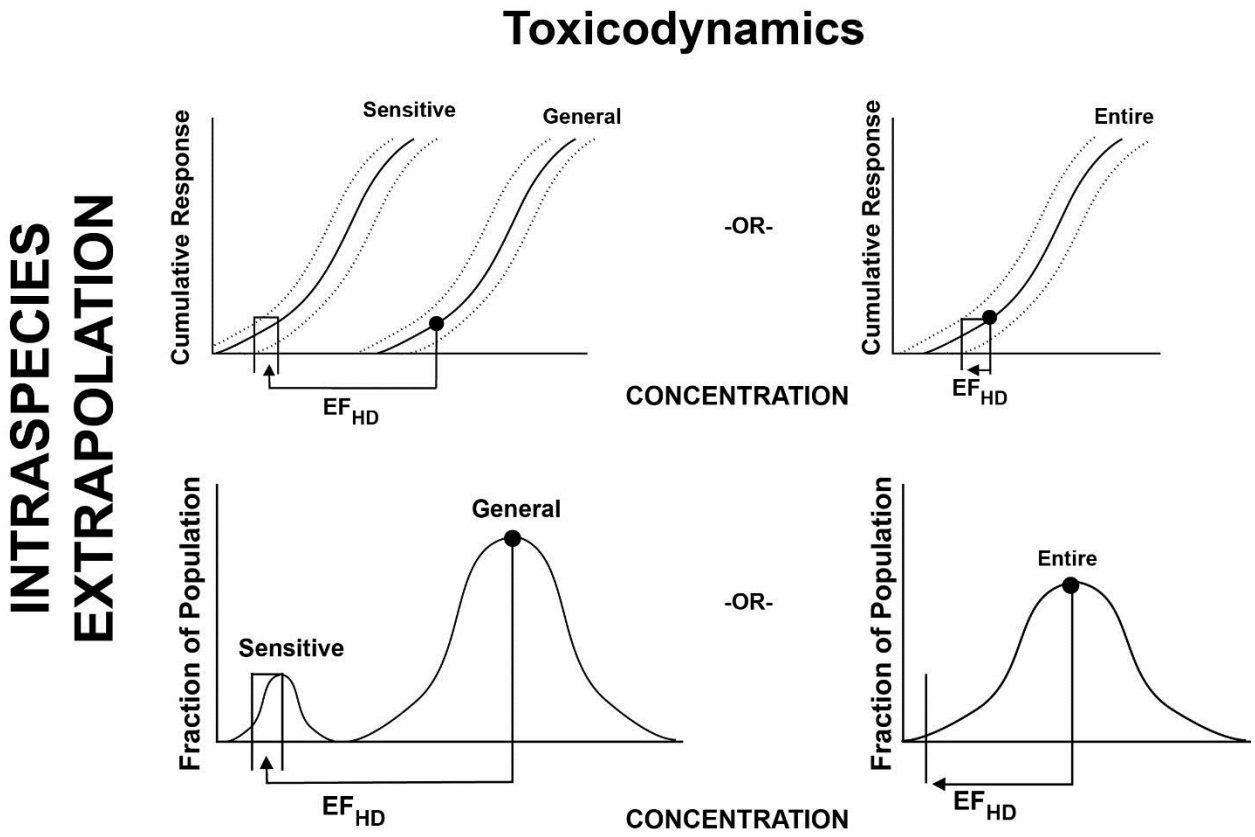

Figure 7. Intraspecies toxicodynamics. Sensitivity may be distributed among the human population in a bi- (or multi-) modal or a unimodal fashion. Even when an identifiable population may be presumed or demonstrated to be sensitive, the distribution of dose metric values may be unimodal. Differences in distribution type affect quantitative methods. TD differences are quantified on the basis of differences in concentration producing the same level of the observed response.

4.3.3. Conclusions for Intraspecies Toxicodynamic Extrapolation Factor

A BBDR or other TD model provides the most biologically appropriate approach for developing an EF_{HD} value. When using empirical ratios, EF_{HD} is the ratio of the concentration producing the specified level of response in sensitive populations to the concentration of the toxicant producing the same level of response at the central tendency of the general or the entire human population. Increased confidence in EF_{HD} is developed when the range of concentrations

INTRASPECIES EXTRAPOLATION OF RESPONSE

For the toxicodynamics component of EF_{HD}, the relationship between the response measured *in vitro* and the response observed *in vivo* should be presented, and EF_{HD} is calculated as the ratio of concentrations producing the same level of the response in the respective population groups—general or entire to sensitive population groups.

used to determine EF_{HD} is comparable to target tissue concentrations at or near the human POD (the animal POD adjusted by the value of the UF_A). Quantitatively, EF_{HD} cannot be less than 1.

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

5. CALCULATION OF THE DATA-DERIVED EXTRAPOLATION FACTOR

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

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

where,

CF = composite factor

EF_{AK} = interspecies TK extrapolation factor

EF_{AD} = interspecies TD extrapolation factor

EF_{HK} = intraspecies TK extrapolation factor

EF_{HD} = intraspecies TD extrapolation factor

In practice, data may only be available to develop a DDEF for one component of extrapolation or another (e.g., data for EF_{AK} but not EF_{AD}). In these situations, the remaining extrapolation is done by an appropriate default procedure. When default values are used, DDEFs and default values (i.e., UFs) are used in combination. Often this default will be one-half order of magnitude UF—as described in the Agency's RfC methodology (U.S. EPA, 1994). When data are not available to develop DDEFs for either component of inter- or intraspecies extrapolation, the default approach (e.g., $BW^{3/4}$ scaling for interspecies extrapolation for oral RfDs, RfC default for interspecies extrapolation for RfCs, or application of UFs) is employed (U.S. EPA, 2011).

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

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APPENDIX A

CASE STUDIES TO ACCOMPANY DATA-DERIVED EXTRAPOLATION FACTOR GUIDANCE

Office of the Science Advisor
Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC 20460

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

LIST OF ABBREVIATIONS

AChE	acetylcholinesterase
AML	amount of metabolite in the liver
AUC	area under the curve
BAA	2-butoxyacetic acid
BMD	benchmark dose
BMD_x	benchmark dose corresponding to an *x*% increase in response
BMDL	benchmark dose lower bound (i.e., lower confidence bound on benchmark dose)
$BMDL_x$	lower confidence bound on benchmark dose corresponding to an *x*% increase in response
BMR	benchmark response
CMG	common mechanism group
CRA	cumulative risk assessment
DAF	dosimetric adjustment factor
DDEF	data-derived extrapolation factor
DMA	dimethyl arsenic acid
EF	extrapolation factor (optional subscripts $_A$ = interspecies/animal-to-human; $_H$ = intraspecies/within human variability; $_D$ = toxicodynamic component; $_K$ = toxicokinetic component)
EF_{AD}	interspecies toxicodynamic EF
EF_{AK}	interspecies toxicokinetic EF
EF_{HK}	intraspecies toxicokinetic EF
EGBE	ethylene glycol monobutyl ether
EPA	U.S. Environmental Protection Agency
GFR	glomerular filtration rate
HED	human equivalent dose
IRIS	Integrated Risk Information System
LC_{50}	lethal concentration for 50% of the population
LOAEL	lowest-observed-adverse-effect level
MCV	mean corpuscular volume
MeHg	methylmercury
MOA	mode of action
MOE	margin of exposure
NMC	N-methyl carbamate
NOAEL	no-observed-adverse-effect level
OPP	Office of Pesticide Programs
PBPK	physiologically based pharmacokinetic
POD	point of departure
RBC	red blood cell
RED	registration eligibility decision
RfD	reference dose
RPF	relative potency factor
SD	standard deviation(s)
TD	toxicodynamic
TK	toxicokinetic

LIST OF ABBREVIATIONS (continued)

UF	uncertainty factor (optional subscripts $_A$ = interspecies/animal to human; $_H$ = intraspecies/within human variability; $_D$ = toxicodynamic component; $_K$ = toxicokinetic component)
UF_A	interspecies UF
UF_H	intraspecies UF
UF_{AD}	interspecies UF for the TD component
UF_{HD}	intraspecies UF for the TD component
UF_{AK}	interspecies UF for the TK component
UF_{HK}	intraspecies UF for the TK component
VC	vinyl chloride

APPENDIX A.
CASE STUDIES TO ACCOMPANY
DATA-DERIVED EXTRAPOLATION FACTOR GUIDANCE

This document is an Appendix to the document *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation*. It presents examples where the availability of data for given chemicals support the derivation of nondefault values for components of uncertainty factors (UFs). Each of the chemicals examined has an existing Integrated Risk Information System (IRIS) file and/or U.S. Environmental Protection Agency (EPA) Program Office risk assessment, although the derivation of data-derived extrapolation factors (DDEFs) contained in these examples may not be found in the those documents. The intent of this case study document is to present examples that instruct the calculation of DDEF values; reference values derived in these case studies should not be used in place of values found in IRIS or Program Office risk assessments.

A consistent format, shown below, has been developed and applied to the case studies. However, different components of case studies are more extensively described for some chemicals than for others. Differences are due to chemical-specific data sets, which may be more informative for some areas than others.

1. Summary
   This section communicates the current assessment(s) (e.g., IRIS, Provisional Peer Reviewed Toxicity Values, Registration Eligibility Decision (RED), premanufacture notice/existing chemical, International Programme on Chemical Safety) and reference values. It includes the individual and composite UFs, and indicates how the DDEF was applied.

2. Hazard Identification and Dose-Response
   This section lists the key studies, identifying the principal study and critical effects including supplemental studies that might help to inform the decision. Methods used to characterize the dose-response relationship and models or data describing response as a function of internal (target tissue) concentration are described. Also included is information on the mode/mechanism of action. The section is not intended to be a compendium of data; rather it should communicate the information necessary to serve as a basis for the case study.

3. Basis for Data-Derived Extrapolation Factor
   This section provides the rationale for developing a DDEF rather than relying on default values. Models or data available for evaluation, the basis for selection of DDEF method, and the connection between the measure of dose and adverse effect are described.

4. Data-Derived Extrapolation Factor Derivation
   This section presents the computation of the DDEF value so that the reader can follow the derivation. Comparisons to other possible values for the UF component are presented.

The case studies illustrate different principles described in the main document. Table A-1 provides a summary of the principles illustrated in each. Note that the Agency does not yet have experience with deriving a DDEF for intraspecies extrapolation with regard to the toxicodynamic (TD) component. As such, no case study for that component is provided here.

Table A-1. Data-derived extrapolation factor case study chemicals and issues

DDEF	Chemical	Other principles or issues
Extrapolation Factor (EF) for the Interspecies/Animal-to-Human (A) Toxicokinetic Component (K) (EF_{AK})[a]	Ethylene glycol monobutyl ether	Choice among dose metrics, physiologically based pharmacokinetic modeling, benchmark dose applied to internal, not external, doses
	Vinyl chloride	Mode-of-action analysis to identify dose metric; internal dose of reactive metabolite in liver chosen as basis for toxicokinetic equivalency between species
EF for the Interspecies/Animal-to-Human (A) Toxicodynamic Component (D) (EF_{AD})	Ethylene glycol monobutyl ether	*In vivo* toxicity evaluations to identify effects to quantify in both species *in vitro*
	Dimethyl arsenic acid	Mode of action analysis, use of *in vitro* data, use of genomics data
	N-methyl carbamate pesticides	Application of a DDEF to a common mechanism group of chemicals, use of DDEF approach to identify data gaps
EF for the Intraspecies/Within Human Variability (H) Toxicokinetic Component (K) (EF_{HK})	Boron	Clearance mechanism identified, surrogate measures of clearance employed for quantitation
	Methylmercury	Toxicokinetic model developed using human data from dietary exposures, choice of dose metric from among several surrogates

[a]The IRIS Assessments for these chemicals calculate a human equivalent dose—based on the results from physiologically based pharmacokinetic modeling. This document guides the development of a DDEF that results in the same human external dose when adjusted for interspecies differences in dosimetry. The DDEF concept applied to interspecies differences in dosimetry makes explicit the magnitude of species differences, whereas methods that calculate a human equivalent dose or human equivalent concentration do not make that calculation explicit.

A.1. INTERSPECIES EXTRAPOLATION FACTOR FOR TOXICOKINETICS

A.1.1. Ethylene Glycol Monobutyl Ether—Interspecies Extrapolation Factor for Toxicokinetics Case Study

A.1.1.1. *Summary*

Ethylene glycol monobutyl ether (EGBE; also known as 2-butoxy ethanol) has an established reference dose (RfD) of 0.1 mg/kg-day in EPA's IRIS database (U.S. EPA, 2010b). This value is based on a human equivalent dose (HED) developed from the results of a chronic inhalation study (NTP, 2000). Internal doses resulting in hemosiderin deposition in liver were extrapolated via physiologically based pharmacokinetic (PBPK) modeling to identify the HED.

As described below, the IRIS RfD is based on a PBPK modeling approach that identified an HED of 1.4 mg/kg-day. To this value, a combined UF of 10 was applied, which comprised a UF value of 10 for variation in sensitivity within the human population/intraspecies UF (UF_H), a value of 1 for the interspecies UF for the toxicokinetic (TK) component (UF_{AK}) (based on application of a PBPK model), and a value of 1 for interspecies toxicodynamic uncertainty factor (UF_{AD}) on the basis of quantified differences in red blood cell (RBC) sensitivity defined *in vitro* [as discussed in the EGBE interspecies/animal-to-human extrapolation factor (EF) for the toxicodynamic (TD) component (EF_{AD}) case study presented later in this appendix].

A.1.1.2. *Hazard Identification and Dose-Response*

No chronic-duration oral studies are currently available for EGBE; there are only two subchronic-duration, 91-day drinking water studies in rats and mice (Dieter, 1993) and a chronic inhalation study in rats and mice (NTP, 2000). Based on a comparison of no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect level (LOAELs) for hematologic and liver effects, rats are clearly more sensitive than mice. Hematologic and hepatocellular changes were noted in both sexes of rats. Hemosiderin accumulation in liver of male rats, a consequence of hemolysis, was chosen as the critical effect. Hemolysis in humans has been demonstrated to result in hemosiderin accumulation in the liver, and acute exposure to EGBE has been shown to produce hematological changes in humans (U.S. EPA, 2010b).

In the 2010 IRIS assessment, PBPK modeling was used to convert each of the inhalation exposures to levels of internal dose, and this measure of dose was combined with response levels. A benchmark dose (BMD) analysis revealed the lower confidence bound on benchmark dose (BMDL) corresponding to a 10% increase in response ($BMDL_{10}$) value, which was chosen as the point of departure (POD). The $BMDL_{10}$ value (expressed as internal dose) was 133 μmol butoxy acetic acid/liter-hour; this was used as the starting point in the development of the IRIS RfD for hemosiderin deposition in male rat liver (NTP, 2000). In the IRIS assessment, the

dose-response relationship was developed by combining PBPK modeling with BMD analysis as shown in Figure A-1.

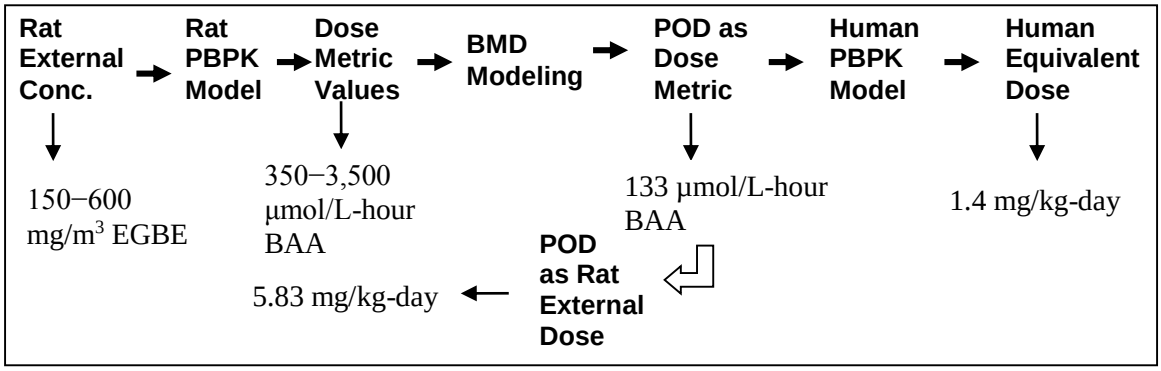

Figure A-1. Analysis approach for developing the human equivalent dose (HED) with values shown.

A.1.1.3. *Basis for Data-Derived Extrapolation Factor*

Area under the curve (AUC) values for the EGBE metabolite 2-butoxyacetic acid (BAA) in arterial blood of male rats following oral exposure were estimated using the PBPK model of Corley et al. (1994) as modified by Corley et al. (1997). BAA levels, rather than EGBE levels, were deemed appropriate measures of exposure based on the findings of Carpenter et al. (1956), who demonstrated *in vitro* that concentrations of 0.1% BAA induced hemolysis; whereas, hemolysis was not induced by EGBE until concentrations of approximately 2.5% were reached. PBPK modeling was used to translate each of the external concentrations to levels of the dose metric, AUC.

Next, BMD modeling was applied to the results of the PBPK modeling. The results of this analysis indicated that an AUC value of 133 µmol/L-hour BAA in arterial blood is the $BMDL_{10}$ for increased hemosiderin deposition in the liver in male rats.

The AUC value for BAA of 133 µmol/L-hour in arterial blood was chosen as the POD for interspecies extrapolation. Next, a human PBPK model was employed to translate this level of the dose metric to an HED of 1.4 mg/kg-day.

A.1.1.4. *Data-Derived Extrapolation Factor Derivation*

The PBPK modeling approach described above was used as the basis for the development of the DDEF. In addition to the data presented in the IRIS file, the rat PBPK model was run to identify that an external dose of 5.83 mg/kg-day in rats would produce this $BMDL_{10}$

concentration (133 μmol/L-hour) of the BAA metabolite (Dr. Richard Corley, personal communication, 2010). The human PBPK model was run to identify the external dose that produced the same level of the dose metric (133 μmol/L-hour); this value of the dose metric was produced by an external dose of 1.4 mg/kg-day in humans. The AUC dose metric was used as the basis for human equivalence.

An alternative approach for deriving the DDEF illustrated in this case study would be to use the ratio of rat-to-human external doses derived from the PBPK model to derive an interspecies toxicokinetic extrapolation factor (EF_{AK}; see eq 1, Section 3.2.2.2.). Using the data described above, a comparison of the external doses would yield an EF_{AK} value of 5.83 mg/kg-day ÷ 1.4 mg/kg-day = 4.2. The 4.2-fold DDEF would replace the default UF_{AK} threefold factor.

Applying the EF_{AK} 4.2-fold[10] to the rat external dose (5.83 mg/kg-day) yields an HED of 1.4 mg/kg-day. This HED is identical to that derived in the IRIS assessment (U.S. EPA, 2010b).

The above methods can also be compared to the default methodology that is based on body-weight scaling (U.S. EPA, 2011). Using a study-specific female rat body weight of 0.188 kg and a default human body weight of 70 kg, these values would result in a default dosimetric adjustment factor (DAF) = $(0.188 \div 70)^{1/4}$ = 0.23. The DAF is multiplied by the animal external dose (5.83 mg/kg-day) to yield an HED of 1.3 mg/kg-day. The reciprocal of the 0.23 DAF is 4.3. When using body weight to ¾ power scaling, the accompanying reduced default interspecies UF is threefold. In this case study, the body-weight scaling default approach yields a similar RfD as the data-derived approaches described above. However, the DDEF approach makes use of quantitative TK data for EGBE and uses the TK/TD framework, which preserves the option also to rely on quantitative TD information.

A.1.1.5. *References for Case Study A.1.1*

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Corley, RA; Markham, DA; Banks, C; Delorme, P; Masterman, A; Houle, JM. (1997). Physiologically based pharmacokinetics and the dermal absorption of 2 butoxyethanol vapors by humans. Toxicol Appl Pharmacol 39: 120-130.

[10]Note: For EGBE, interspecies toxicodynamic data exist and have been used in the IRIS assessment to replace the default factor of 3.2 for UF_{AD} with a value of 1. This case study focused only on TK; please see the EGBE $DDEF_{AD}$ case study in Section A.2.1 of this appendix.

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A.1.2. Vinyl Chloride—Interspecies Extrapolation Factor for Toxicokinetics Case Study

A.1.2.1. *Summary*

Vinyl chloride (VC) has an established oral RfD of 3×10^{-3} (0.003) mg/kg-day in the current IRIS file (U.S. EPA, 2000a). The POD for liver toxicity is a NOAEL value of 0.13 mg/kg-day from a chronic feeding study in male Wistar rats. PBPK modeling was used to develop an HED of 0.09 mg/kg-day, making the UF_{AK} unnecessary. The composite UF of 30 comprises a UF of 3 to cover TD differences between species and a default value of 10 for intraspecies variability. Species differences in dosimetry were determined on the basis of external doses required to produce the same level of the toxicologically active metabolite in the critical organ (liver). This case study demonstrates the application of data to inform EF_{AK}.

A.1.2.2. *Hazard Identification and Dose-Response*

The liver was selected as the critical target organ of VC in humans and experimental animals. Strong epidemiological evidence exists for liver effects in humans. Studies involving workers in the polyvinyl chloride plastics industry from several countries have demonstrated a significant relationship between VC inhalation exposure and liver cancer. While limited evidence may suggest a risk for other, nonliver tumors (e.g., leukemia, brain, lung, pancreas, mammary), "vinylchloride is not likely to be associated strongly with cancers other than liver in humans" (U.S. EPA, 2000a). Other noncancer effects noted in epidemiologic investigations include impaired liver function and biochemical and histological evidence of liver damage and focal hepatocellular hyperplasia. Pulmonary function appeared unimpaired, and no solid evidence of teratogenicity in humans has been identified.

Limited data are available for inhalation studies in animals. Data are available from a 12-month inhalation study, which supports the liver as the critical organ. Bi et al. (1985) exposed Wistar rats to 0, 10, 100, or 3000 ppm VC for 6 hours/day, 6 days/week. Cellular alterations, degeneration, and necrosis were observed in the seminiferous tubules of the testes, with a NOAEL in the 10-ppm exposure group. This same exposure was the LOAEL for liver effects, characterized only as liver weight changes.

Like the inhalation results, studies conducted via the oral route identify the liver as the critical target organ. Til et al. (1991; 1983) reported the results of two-year rodent bioassays with VC in feed. Groups of 100 or 50 male and female Wistar rats were exposed to 0, 0.014, 0.13, or 1.3 mg/kg-day in feed for only 4 hours/day to minimize volatilization. The VC content of feed was measured before and after feeding to control for volatilization. Multiple hepatic effects were noted, including several that were deemed neoplastic or preneoplastic. The pathologists were able to delineate and determine incidences for two effects not thought to represent neoplastic or preneoplastic changes. Liver cell polymorphisms and proliferative bile duct epithelium cysts served as the basis for identifying the liver as the critical target tissue for noncancer effects in the chronic bioassay. These same changes were observed in a second study, but the doses employed in that study (Feron et al., 1981) were higher than those employed by Til and coworkers. Because of a lack of confidence in the outcome from a BMD modeling approach based on external dose, a traditional (i.e., NOAEL) approach to dose-response evaluation for events not associated with carcinogenicity was used. The POD for species extrapolation was the NOAEL of 0.13 mg/kg-day for liver cell polymorphisms and bile duct cysts.

The initial process in the mode of action (MOA) appears to be the formation of reactive and short-lived metabolites that achieve only low steady-state concentrations. These metabolites are thought to be responsible for the toxic effects of VC (Bolt, 1978). Experiments that manipulated the longevity of cytochrome P450-derived metabolites demonstrated an inverse relationship between metabolite longevity and protein and nucleotide binding (Guengerich et al., 1981). Thus, the metabolism of VC to reactive intermediates was demonstrated to be a critical determinant of toxicity. Because of the short-lived nature of the metabolite(s), a measure of their concentration in the target tissue (liver, the site of their formation) was deemed the appropriate dose metric for quantitative application. This concept and approach has also been applied to methylene chloride (Andersen et al., 1987) and chloroform (ILSI, 1997).

A.1.2.3. *Basis for Data-Derived Extrapolation Factor*

The liver was determined to be the target organ, and evidence indicated that the formation of a reactive metabolite was likely responsible for the toxicity of VC. BMD modeling of external doses failed. Because of this, the POD for extrapolation was determined as the study

NOAEL for liver effects, 0.13 mg/kg-day. Consistent with the guidance for reference concentration derivation, PBPK modeling of the formation of the active metabolite in liver was used as the basis for determining an HED. PBPK models were developed for rats and humans and used to extrapolate dosimetry between species (Clewell et al., 1995b; Clewell et al., 1995a); the models were subjected to an external peer review and deemed sufficient for quantitative reliance. The NOAEL dose (0.13 mg/kg-day) was converted into the dose metric for VC—the amount of metabolite in the liver (AML), with units of concentration (mg/L) of liver.

A.1.2.4. *Data-Derived Extrapolation Factor Derivation*

Importantly for dose extrapolation, these models demonstrated a linear relationship between applied dose and the dose metric (i.e., AML) up to doses approximating 25 mg/kg-day. This allowed linear interpolation to be used to identify levels of external doses associated with specific amounts of the internal dose, rather than specific iterations via PBPK modeling. The rat NOAEL dose of 0.13 mg/kg-day produced AML at a value of 3.0 mg/L (see Figure A-2). This level of internal exposure in the 70-kg human was determined to result from a drinking water exposure of 0.09 mg/kg-day (the HED). Thus, doses of 0.13 mg/kg-day and 0.09 mg/kg-day in the rat and human, respectively, are toxicokinetically equivalent.

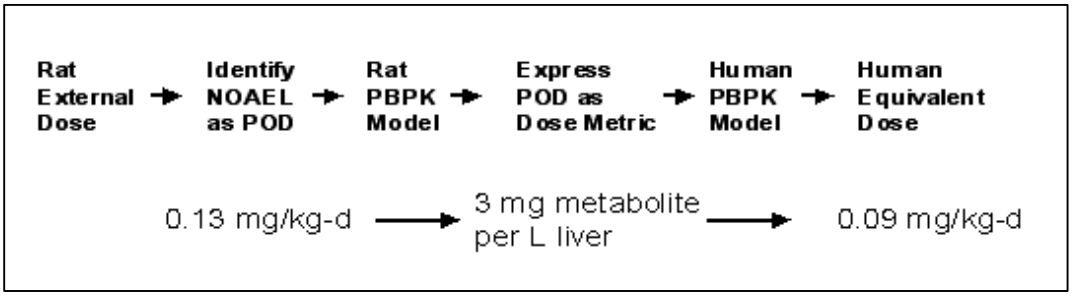

Figure A-2. Schematic for dose extrapolation for vinyl chloride. The POD was defined as the external dose in the rat, with PBPK modeling of the rat. PBPK modeling of the dose metric translated dose from units of applied dose to units of tissue concentration, representing the dose metric. PBPK modeling in the human identified the HED, the dose producing the same level of the dose metric in the rat study at the POD.

The IRIS file for VC used the HED as the POD to which UFs are to be applied. An alternative approach illustrated in this case study to deriving the DDEF for interspecies TK differences would be to use the ratio of rat and human external doses resulting in the same level of target tissue exposure at the POD.

Using the data described above, a DDEF value for EF_{AK} of 0.13 ÷ 0.09 = 1.44 is indicated. Combined with the default value of 3 for TD, a DDEF for EF_{AK} would be 3 × 1.44 = 4.32, in place of the default value of 10. By applying the DDEF calculated above (4.32-fold) and the default value of 10 for UF_H to the animal NOAEL (0.13 mg/kg-day), the RfD is 0.13 mg/kg-day ÷ 43.2 = 0.003 mg/kg-day. This is the same value that was calculated for the IRIS assessment, which was expressed as the HED/UF, or 0.09 mg/kg-day/30 (U.S. EPA, 2000a).

The above methods can also be compared to the default methodology that is based on body-weight scaling (U.S. EPA, 2011). Using a default value of 0.462 kg for adult Wistar rats (U.S. EPA, 1988) and applying the body-weight scaling approach described in *Recommended Use of Body Weight $^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011) to the NOAEL of 0.13 mg/kg-day, an HED can be calculated as:

$$0.13 \text{ mg/kg-day} \times 0.462 \text{ kg} = 0.060 \text{ mg}$$

$$0.06 \text{ mg} \times (70 \text{ kg} \div 0.462 \text{ kg})^{3/4} =$$

$$0.06 \text{ mg} \times 43.19 = 2.59 \text{ mg}$$

$$2.59 \text{ mg} \div 70 \text{ kg} = 0.037 \text{ mg/kg-day} \qquad \text{(A-1)}$$

In this case study, the body-weight scaling default approach yields a slightly lower POD value than the data-derived approaches described above. Furthermore, this approach makes use of quantitative data on VC.

A.1.2.5. *References for Case Study A.1.2*

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ILSI (International Life Sciences Institute). (1997). An evaluation of EPAs proposed guidelines for carcinogen risk assessment using chloroform and dichloroacetate as case studies. Report of ILSI HESI Expert Panel. Washington, DC.

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A.2. INTERSPECIES EXTRAPOLATION FACTOR FOR TOXICODYNAMICS

A.2.1. Ethylene Glycol Monobutyl Ether—Interspecies Extrapolation Factor for Toxicodynamics Case Study

A.2.1.1. *Summary*

As noted in Section A.1.1.1, EGBE has an established RfD of 0.1 mg/kg-day in EPA's IRIS database (U.S. EPA, 2010b). This value is based on the results of a subchronic-duration drinking water study in mice and rats where increases in Kupffer cell hemosiderin content secondary to hemolysis were determined to be the critical effect. Derivation of the HED of 1.4 mg/kg-day is detailed in the EGBE TK case study in Section A.1.1 and in the IRIS file for EGBE (U.S. EPA, 2010b).

The IRIS RfD is based on a PBPK modeling approach that identified an HED of 1.4 mg/kg-day. UFs were applied to this POD value to derive the RfD. A total UF of 10 was applied to this HED to derive the RfD. A default value of 10 was used to account for variation in sensitivity within the UF_H. The interspecies UF (UF_A) was reduced to 1 on the basis of dosimetry adjustments to account for TK (discussed case study A.1.1, for EGBE TK). EF_{AD} was also reduced to a value of 1, because studies indicate that humans may be significantly less sensitive than rats to the hematological effects of EGBE. *In this case study, however, issues associated with EGBE TD are described and an alternative approach to DDEF derivation based on the use of in vitro data are described.*

A.2.1.2. *Hazard Identification and Dose-Response*

Based on extensive review of the literature, hematologic effects appear to be the most sensitive of the adverse effects observed in laboratory animals exposed to EGBE. Hematologic effects (e.g., hemoglobinuria) have also been documented in worker populations exposed to technical grade EGBE (Cellosolve™) and following ingestion of cleaning products containing EGBE (U.S. EPA, 2010b).

Key events in the proposed MOA in RBCs leading to increases in hemoglobin accumulation in Kupffer cells include:

- Oxidative metabolism to BAA
- RBC swelling and lysis (probably preceded by an increase in osmotic fragility and loss of deformability)

- Decreased RBC count, hemoglobin, and hematocrit, and in response, increased production of immature RBCs (reticulocytes) by the bone marrow

Carpenter et al. (1956) incubated RBCs from rats, mice, rabbits, monkeys, dogs, humans, and guinea pigs with 0.1% BAA. Results demonstrating maximum time without hemolysis were 35−40, 40−45, 60−90, 103−120, 80−120, 147−268, and 360 minutes, respectively. These data indicate that guinea pigs and humans are more resistant and that rats and mice may be more sensitive to the hemolytic effects of BAA. Studies with Cellosolve and BAA demonstrated that RBCs were much more sensitive to BAA than to Cellosolve. These results led Carpenter et al. (1956) to speculate that the BAA metabolite of Cellosolve was responsible for hemolysis.

In a direct comparison of the effects of BAA on rat and human RBCs, Udden and Patton (1994) devised a study using filtration, phase contrast light microscopy, and routine hematologic methods. RBCs were obtained from healthy adults via venipuncture and from 9- to 11-week-old male Fischer 344 rats via cardiac puncture. Rat RBCs were incubated with 0.2 and 2.0 mM BAA; human RBCs were incubated with only 2.0 mM BAA. Rat RBC demonstrated 30% hemolysis after incubation with 2.0 mM BAA for 4 hours, and 4% hemolysis after incubation with 0.2 mM BAA for 6 hours or longer. The 4% measure was "mild," and the data were not shown. Incubation of human RBC with 2.0 mM BAA for 4 hours resulted in no increase in hemolysis over the background (control) level of 1%. Histologic evaluations of rat, but not human, RBC preparations postexposure demonstrated RBC "ghosts," which are cell membranes from lysed cells. These incubation conditions resulted in increases in mean corpuscular volume (MCV) in rat RBCs incubated with 0.2 mM BAA, but not human RBCs incubated with 2.0 mM BAA. The increase in MCV indicates RBC swelling, an event that leads to fragility.

Finally, these investigators examined the deformability of RBCs by pumping them through a narrow-diameter filter and monitoring an increase in inflow pressure. The results were presented graphically, as pressure versus time plots. The logic behind this study was that RBCs normally can deform to pass through tight spaces (like capillaries). However, RBCs in which swelling has been induced and in which other mechanisms may have been activated that can lead to increased membrane rigidity, will not pass through the membrane, resulting in an increase in inflow pressure. Rat RBCs demonstrated a nearly doubled pressure in rat RBCs incubated with 0.2 mM BAA for 4 hours, and a roughly tripled pressure when incubated with 2.0 mM BAA for 4 hours compared to controls. In contrast, pressure differences between human RBCs incubated in the absence of BAA and in the presence of 2.0 mM BAA for 4 hours were not distinguishable. Udden and Patton (1994) indicated that their findings that human RBCs were less sensitive *in vitro* to the hemolytic effects of BAA were consistent with multiple other findings. In a follow-up study, Udden (2002) demonstrated again that human RBCs were less susceptible to the

effects of BAA than RBCs from rats. Whereas rat RBCs demonstrated alterations including deformability and changes in MCV at 0.05 mM, human RBCs did not demonstrate changes in MCV at concentrations below 10 mM—a difference of 200-fold.

Ghanayem and Sullivan (1993) also performed a species comparison of BAA-induced RBC effects *in vitro*. An advantage of this study is that it applied a pairwise statistical design to determine the effect of treatment over control. Male Fischer 344 rats 15 weeks of age were used, and human blood was drawn from adult male donors. BAA (2.0 mM incubated up to 4 hours) produced only slight, but not statistically significant, alterations of hematological parameters: hematocrit was increased 2−4% and MCV was increased less than 4% above vehicle controls. The authors concluded that their results demonstrated that humans were "minimally sensitive" to the *in vitro* effects of BAA. In comparison, RBCs from rats were deemed "relatively susceptible" to the effects of BAA. A graphic presentation of data demonstrated that in rats, MCV and hematocrit were increased nearly 60% when exposed to 2.0 mM BAA.

Udden (2002) compared the subhemolytic and hemolytic effects of BAA in rat and human RBCs. RBC deformability, density, MCV, count, osmotic fragility, and hemolysis were measured following a 4-hour exposure. Alterations were noted in loss of deformability, but at concentrations that were 150-fold higher in rat RBCs than in human RBCs (0.05 mM vs. 7.5 mM). A larger species difference in response was noted in comparing effect levels for increases in MCV—these differences approximated 200-fold, with rat RBCs responding at 0.05 mM and human RBCs demonstrating alterations of MCV at 10 mM. Changes in osmotic fragility were similar.

A.2.1.3. *Basis for Data-Derived Extrapolation Factor*

Several data sets are available in which the responsiveness of rat and human blood at 2.0 mM can be compared. *However, comparison of effect data for TD is most appropriately accomplished by comparing the different concentrations resulting in the same response.*

Hemolytic effects were observed in rat RBCs exposed to BAA at concentrations as low as 0.05 mM. In contrast, similar hemolytic effects were observed in human blood exposed to 10 mM BAA. The IRIS file indicates that humans may be much less sensitive than rats to the hematologic effects of EGBE, and for this reason a value of 1 was selected for the UF_{AD}.

A.2.1.4. *Data-Derived Extrapolation Factor Derivation*

One potential approach would be to derive an adjustment factor of 0.005 for UF_{AD} (0.05 mM/10 mM = 0.005). This approach requires the assumption that the TD differences observed *in vitro* would be approximated *in vivo*. Uncertainty about this issue prompted EPA to

take a different approach. In the IRIS assessment (U.S. EPA, 2010b), the UF value for UF_{AD} was established at 1.

Regarding TD, *in vivo* (Carpenter et al., 1956) and *in vitro* (Udden, 2002; Udden and Patton, 1994; Udden, 1994; Ghanayem and Sullivan, 1993) studies indicate that humans may be significantly less sensitive than rats to the hematological effects of EGBE. For this reason, a value of 1 was selected for the TD portion of the UF_A.

A.2.1.5. *References for Case Study A.2.1*

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A.2.2. Dimethyl Arsenic Acid—Interspecies Extrapolation Factor for Toxicodynamics Case Study

A.2.2.1. *Summary*

Dimethyl arsenic acid (DMA), also known as cacodylic acid, is an herbicide used on primarily cotton and turf. DMA is also a urinary metabolite in most mammals, including humans, following direct exposure to inorganic arsenic. The Office of Pesticide Programs (OPP), in collaboration with National Health and Environmental Effects Research Laboratory scientists, developed a nonlinear MOA assessment for DMA for the development of rat bladder tumors (U.S. EPA, 2006b). The MOA and dose-response assessments were developed using the MOA (Sonich-Mullin et al., 2001) and Human Relevance (Boobis et al., 2006) Frameworks. The information provided by the MOA analysis also provides the basis for the chronic RfD and

the respective UFs in the RED for cacodylic acid. Instead of the default 10-fold factor to extrapolation from animal to human, OPP has reduced the TD component of UF_A (EF_{AD}) to a value of 1. This case study describes the determination of the one-half order of magnitude (threefold) factor, which was used to account for TD differences between animals and humans. The default value of 10 for UF_H was also applied leading to a composite factor of 30.

A.2.2.2. *Hazard Identification and Dose-Response*

In rat carcinogenicity studies, oral exposure to DMA^V leads to bladder tumors. The overall weight of the evidence provides convincing support for a nonlinear MOA for DMA^V-induced carcinogenesis in rodents. The key events include:

- Reductive metabolism of DMA^V to DMA^{III}.
- DMA^{III} causes urothelial cytotoxicity. Regenerative cell proliferation then ensues in order to replace dead urothelial cells. The amount of cell killing is a function of the severity of the cytotoxicity, which is related to the amount of DMA^{III} present. The amount of DMA^{III} is dependent on the conversion of DMA^V to DMA^{III}.
- Sustained cytotoxicity leads to regenerative cell proliferation, which in turn, ultimately leads to hyperplasia and bladder tumors.

To obtain a tumor via the proliferation/replication genetic error process, induced cell proliferation needs to be persistent. There is convincing experimental evidence to indicate that this is the case for the rat bladder. There is a clear association of DMA^V treatment and cell killing/regenerative proliferation and bladder tumors. The amount of proliferation would be a function of the amount of cell killing since the tissue will undergo regenerative proliferation in response to cell killing. As the severity of cytotoxicity increases with increasing levels of DMA^V (DMA^{III}), regenerative proliferation is the rate limiting step for tumor formation, even though the product is chromosome mutations. Thus, a tumor dose-response curve would be influenced by the induced cell proliferation curve, even though chromosomal mutations may be an output. DMA^V-induced tumors would be produced only at treatment durations and dose levels that result in significant cell killing and regenerative cell proliferation in the urothelium of the bladder. Experimental data are available to support the coincidence of key events at similar concentration levels. The levels of DMA^{III} in the urine of rats treated with 100-ppm DMA^V range from 0.5−5.0 μM. The lethal concentration for 50% of the population (LC_{50}) values for DMA^{III} in rat and human urinary epithelial cells *in vitro* are 0.5−0.8 μM. A significant increase in chromosome aberrations occurs in human lymphocytes *in vitro* at about 1.35 μM DMA^{III}. At

100 ppm, there is significant cell killing and regenerative proliferation in female rat bladders. It appears that chromosomal mutations, cytotoxicity, and cell proliferation can potentially occur concurrently at 100-ppm DMA^V, which is the tumorigenic dose in female rats via diet.

Among the several key events, all of which are necessary for tumor formation, cell proliferation has been used for deriving a POD because it is needed for increasing the likelihood of chromosome mutation formation and for the perpetuation of genetic errors, as well as for hyperplasia. A $BMDL_{10}$ value (0.43 mg/kg bw/day) is the basis for the POD in deriving an RfD or a margin of exposure (MOE). This approach is considered public health protective because a $BMDL_{10}$ of 0.43 mg/kg bw/day is approximately an order of magnitude lower than the dose (~0.7 mg/kg bw/day or 10 ppm) that resulted in a 1.5-fold statistically nonsignificant increase in cell proliferation after 10 weeks of exposure to DMA^V and about two orders of magnitude lower than the dose (~9.4 mg/kg bw/day) resulting in neoplasia in the feeding studies.

A.2.2.3. *Basis for Data-Derived Extrapolation Factor*

In the 2006 DMA risk assessment (U.S. EPA, 2006b), instead of the default 10-fold factor to extrapolate from animal to human, the OPP reduced the TD component to 1. A factor of 3 was used to account for interspecies differences in TK. UF_{AD} was reduced to 1 given that, at a similar dose at the target site (i.e., bladder urothelial), humans and rats are expected to respond pharmacodynamically similar. This case is built on a combination of information:

- Chemical-specific *in vitro* data from Cohen et al. (2002) which show that human and rat cells respond similarly to exposure to DMA^{III}—the LC_{50} values for cytotoxicity in human and rat epithelial cells were very similar (0.8 μM and 0.5 μM, respectively).
- There is microarray support (Sen et al., 2005). Qualitatively the genes that are upregulated in human urinary bladder epithelial cells (UROtsa) are similar to those upregulated in rat urinary bladder epithelial cells (MYP3) exposed to DMA^V *in vitro*. In this study, the rat cell line was quantitatively more sensitive compared to the human cell line.
- General information on the development and function of the bladder along with incidence of bladder tumors in human populations qualitatively supports the animal MOA in humans.

There are known pharmacokinetic differences between rats and humans. These pharmacokinetic differences include sequestration of DMA^{III} by rat hemoglobin, which results in a longer retention time in the rat compared to humans or mice, and the increased urinary output of trimethylarsine oxide in rats compared to humans. Because of uncertainties regarding

quantifying the tissue dose in humans using rat data, and in the absence of a fully developed PBPK model at the time of the risk assessment, an EF_A of 3 was applied. Pharmacokinetic analyses indicate that, for similar chronic low-level exposures, rats would take longer to achieve steady-state concentrations of DMA^V and metabolites in target tissue compared to humans, and that for a given exposure target tissue, concentrations would be elevated for a longer time after exposure ceased in the rat because rat hemoglobin acts as a slow-release storage depot. Note that the half-life in the rat for DMA^V appears to correlate with erythrocyte half-life, indicating that the binding to hemoglobin is not readily reversible. There are, however, uncertainties regarding the quantitative differences between rats and humans that prevent further reduction of the UF_A.

A.2.2.4. *Data-Derived Extrapolation Factor Derivation*

A value of 1 was developed for EF_{AD} based on the rationale above in combination with the animal MOA and implementation of the Human Relevance Framework. Together, the weight of the evidence provides a strong case for TD equivalence between rats and humans. A factor of 3 was used to account for interspecies differences in TK. The default value of 10 for UF_H was also applied, leading to a composite factor of 30.

A.2.2.5. *References for Case Study A.2.2*

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A.2.3. N-methyl Carbamate Pesticides—Interspecies Extrapolation Factor for Toxicodynamics Case Study

A.2.3.1. *Summary*

OPP released its revised cumulative risk assessment (CRA) for the N-methyl carbamates (NMCs) in 2007 (U.S. EPA, 2007). As required under the Food Quality Protection Act (FQPA) (FQPA, 1996), a CRA incorporates exposures from multiple pathways (i.e., food, drinking water, and residential/nonoccupational exposure to pesticides in air, or on soil, grass, and indoor surfaces) for those chemicals with a common mechanism of toxicity (FQPA, 1996). This CRA began with the identification of a group of chemicals, called a common mechanism group (CMG), which induces a common toxic effect by a common mechanism of toxicity. The NMCs were considered to be a CMG due to their common inhibitory actions on acetylcholinesterase (AChE), an enzyme that is normally required for ending cholinergic transmission in the nervous system.

This case study describes a sensitivity analysis conducted in the risk characterization phase of the revised CRA using a DDEF approach. TD equivalence for animal-to-human extrapolation ($EF_{AD} = 1$) was assumed for several chemicals for which human data were not available.

A.2.3.2. *Hazard Identification and Dose-Response*

The NMCs were established as a CMG by EPA in 2001 (U.S. EPA, 2001a) based on their similar structural characteristics and shared ability to inhibit AChE by carbamylation of the serine hydroxyl group located in the active site of the enzyme. When AChE is inhibited, acetylcholine accumulates and results in cholinergic toxicity, due to continuous stimulation of cholinergic receptors throughout the central and peripheral nervous systems that innervate virtually every organ in the body. An important aspect of NMC toxicity is the rapid nature of the onset and recovery of effects; following maximal inhibition of cholinesterase (typically between 15 and 45 minutes), recovery occurs rapidly (minutes to hours).

Inhibition of AChE is considered the first and critical step in the toxicity of NMCs. Human health monitoring has capitalized on the availability of blood cholinesterase measurements, and these have been widely accepted as a marker of exposure. However, since the brain may be considered more as the critical target site, data on inhibition of brain AChE are obviously only available using laboratory animals. Brain AChE data have been widely used as a POD for risk assessment.

EPA used the relative potency factor (RPF) method to determine the combined risk associated with exposure to NMCs. Briefly, the RPF approach uses an index chemical as the

point of reference for comparing the toxicity of the NMC pesticides. RPFs are calculated as the ratio of the toxic potency of a given chemical to that of the index chemical and are used to convert exposures of all chemicals in the group into exposure equivalents of the index chemical. Because of high-quality dose-response data for all routes of exposure, as well as high-quality time-to-recovery data, EPA selected oxamyl as the index chemical for standardizing the toxic potencies and calculating RPFs for each NMC pesticide.

A.2.3.3. ***Basis for a Data-Derived Extrapolation Factor***

In the single chemical assessments for most NMCs, a default value of 10 for animal-to-human/intraspecies extrapolation factor (EF_H) was used. For three NMCs (aldicarb, methomyl, and oxamyl), however, studies in human subjects were determined by EPA to be ethically and scientifically acceptable for use in risk assessment, after considering the advice of the Human Studies Review Board. These studies were used to derive the chemical-specific EF_A for these three chemicals. Table A-2 summarizes the dose-response and time-course modeling data for critical rat and human studies for these three NMCs.

Table A-2. N-methyl carbamate cumulative risk assessment: interspecies/animal-to-human extrapolation factors and corresponding rat and human BMD_{10}s and $BMDL_{10}$s

Chemical	Rat						Human			UF_A
	Brain			RBC			RBC			
	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)	Half-life (hr)	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)	Half-life (hr)	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)	Half-life (hr)	
Aldicarb	F = 0.048 M = 0.056	F = 0.035 M = 0.035	1.5	0.031	0.020	1.1	0.016	0.013	1.7	2
Methomyl	0.486	0.331	1.0	0.204	0.112	0.8	0.040	0.028	1.6	5
Oxamyl	F = 0.145 M = 0.185	F = 0.111 M = 0.143	0.9	0.278	0.158	0.8	0.083	0.068	2.4	3

With regard to the EF_{AK}, NMCs have similar metabolic profiles across species. NMCs do not require activation; the parent compound is an active AChE inhibitor. Although some metabolites of NMCs have been shown to be active as well, none have been shown to be more potent than the parent chemical. Thus, metabolism is considered to be a detoxification process. As such, species differences in tissue dosimetry are likely correlated with differences in body weight to the ¾ power (U.S. EPA, 2011).

The mechanism of toxic action of NMCs is reproducible across a range of species, including rodents and humans. In addition, the AChE enzyme in humans and rats has similar function and structure. See reviews by Radić and Taylor (2006) and Sultatos (2006). The half-life to recovery values[11] for rats and humans provided in Table A-2 range from approximately 1 to 2 hours and demonstrate the similarity of the half-lives of the two species. Based on this information, given a similar dose or concentration at the target site, it is likely that human and rat AChE would respond similarly. This understanding can inform the interspecies DDEF (EF_{AD}).

A.2.3.4. *Data-Derived Extrapolation Factor Derivation*

For the CRA, toxic potencies for the NMCs were determined using brain AChE inhibition measured at peak inhibition following gavage exposures in rats. The Agency used an exponential dose-time-response model to develop BMD estimates at a level estimated to result in 10% brain cholinesterase inhibition (i.e., a BMD or BMD_{10}) to estimate RPF. Ratios comparing doses (administered) that produce the same magnitude of effect may then be derived. Using the data in Table A-2, and dividing the $BMD_{10\text{-rat}}$ by the $BMD_{10\text{-human}}$ for RBC cholinesterase inhibition:

$$\text{Aldicarb: } 0.031 \div 0.016 = 1.9 \approx 2 \quad \text{(A-2)}$$

$$\text{Methomyl: } 0.204 \div 0.040 = 5.1 \approx 5 \quad \text{(A-3)}$$

$$\text{Oxamyl: } 0.278 \div 0.083 = 3.3 \approx 3 \quad \text{(A-4)}$$

This analysis showed that the ratio of the BMDs for rat/human ranges from 2 to 5 for these NMCs. This range would tend to support the DDEF approach described here to reduce the standard interspecies factor value from 10 to 3. The concentration of toxicant at the active site *in vivo* is controlled by TK processes. The available data described the TD processes (enzyme regeneration) and indicated that rats and humans were very similar in this area. The remaining threefold (default) value for UF_A addresses species differences in TK.

[11]Recovery half-life differs from elimination half-life. While elimination half-life is not an acceptable basis for calculation of a DDEF for *toxicokinetic* components, enzyme regeneration measurements (the subject of the present analysis) are also expressed in half-life values. This usage refers to regeneration of enzymatic activity or *de novo* synthesis of additional enzyme (protein). In this instance, "half-life" measures are an adequate basis for derivation of DDEF values for *toxicodynamic* events.

It may be possible to use *in vitro* studies using human and rat tissues and human and rat AChE to test this hypothesis. In other words, it may be possible to use *in vitro* studies to demonstrate TD equivalence between rats and humans. If these data were available and they showed TD equivalence, the Agency could reduce the interspecies factor for those NMCs to a value of 3 without human toxicity studies. Due to the lack of these *in vitro* studies, the Agency does not believe it appropriate at this time to refine the standard for EF_A of 10 further. Instead, the Agency has used the DDEF approach as a sensitivity analysis in its risk characterization.

In this sensitivity analysis, the interspecies factor was reduced from 10 to 3 based on the assumption of TD equivalence for carbaryl, carbofuran, and formetanate HCl. These three NMCs were identified since they were shown in the CRA to contribute a large portion of the estimated human exposure to the cumulative risk to this group. The results of this sensitivity analysis for the food exposure assessment are shown below in Table A-3. The Agency has used a probabilistic approach to the food exposure assessment. The estimated exposures of the NMC as a group (i.e., oxamyl equivalents) and the MOE at the 99.9th percentile are shown here. The target MOE is 10 or higher for EF_H. The table compared the MOEs when using the standard UF to those obtained with the modified UF_A.

Table A-3. Sensitivity analyses N-methyl carbamate cumulative food assessment: Data-derived extrapolation factor approach for interspecies uncertainty factors

Age group		Estimated exposure at the 99.9th percentile (mg/kg oxamyl equivalents)	MOE at the 99.9th percentile	Percentile at which target of 10 is reached
Baseline CRA	Children 1−2	0.0229	7.9	99.848th
	Children 3−5	0.0209	8.6	99.870th
DDEF approach for interspecies UF	Children 1−2	0.0183	9.8	99.896th
	Children 3−5	0.0171	10.5	N/A

Review of this table shows that:

- Using the standard interspecies factor of 10 for all NMCs without human data, the (baseline) MOEs at the 99.9th percentile of exposure are less than 10 for children 1−2 and 3−5 years of age. Furthermore, MOEs reach the target of 10 at the 99.848th and 99.870th percentiles of exposures.

- When considering an alternative approach to the interspecies factor that assumes a value of 3 for carbaryl, carbofuran, and formetanate HCl, the MOEs at 99.9th percentile of exposure increase to 9.8 and 10.5 for children 1−2 and 3−5 years of age, respectively—a 20% increase in MOEs. The exposure for the younger age group reaches the target MOE of 10 at the 99.896th percentile of exposures.

This sensitivity analysis suggests that additional data could provide a substantial improvement in the refinement of the CRA.

A.2.3.5. *References for Case Study A.2.3*

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A.3. INTRASPECIES EXPTRAPOLATION FACTOR FOR TOXICOKINETICS

A.3.1. Boron and Compounds—Intraspecies Extrapolation Factor for Toxicokinetics Case Study

A.3.1.1. *Summary*

In the EPA IRIS summary file for Boron and Compounds (U.S. EPA, 2004b), an RfD value of 2×10^{-1} mg/kg-day has been developed based on the critical developmental effect of decreased fetal weights (U.S. EPA, 2004b). The POD for the derivation of this value is the $BMDL_{05}$ value of 10.3 mg/kg-day. The $BMDL_{05}$ is based on the combined results of two separate studies chosen for the derivation of the RfD (Price et al., 1996; Price et al., 1994; Heindel et al., 1992). Using data from rats (Vaziri et al., 2001) and humans (Pahl et al., 2001), a mathematical model was applied to the EF_{AK} to address interspecies TK. The EF_{AK} was calculated to be 3.3. An intraspecies toxicokinetic extrapolation factor (EF_{HK}) of 2.0 was estimated from three studies (Sturgiss et al., 1996; Krutzén et al., 1992; Dunlop, 1981), using glomerular filtration rate (GFR) as a surrogate for boron clearance. The remaining uncertainty in the RfD derivation was from TD. Interspecies and intraspecies TD uncertainty were each assigned the default value of one-half order of magnitude (3.16). The product of all the adjustment and subfactors served as the total adjustment factor of 66. The RfD was derived by dividing the $BMDL_{05}$ of 10.3 mg/kg-day by the adjustment factor and rounding to one digit. This case study demonstrates the use of data to develop an EF_{HK}. Specifically, it demonstrates how a value of 2.0 for EF_{HK} was identified from three studies (Sturgiss et al., 1996; Krutzén et al., 1992; Dunlop, 1981), using GFR as a surrogate for boron clearance.

A.3.1.2. *Hazard Identification and Dose-Response*

Oral animal studies have identified the testes and the developing fetus as the two most sensitive targets of boron toxicity in multiple species (U.S. EPA, 2004b). Testicular effects include reduced organ weight and organ-to-body weight ratio, atrophy, degeneration of the spermatogenic epithelium, impaired spermatogenesis, reduced fertility, and sterility. The mechanism of action for boron's effect on the testes is not known, but the available data suggest an effect on Sertoli cells. Developmental effects following oral exposure to boron have been reported in mice, rabbits, and rats, and include high prenatal mortality, reduced fetal body weight, and malformations and variations of the eyes, central nervous system, cardiovascular system, and axial skeleton. Similarities in the NOAEL values for the reproductive toxicity studies and quality control issues complicated the choosing of testicular effects as the critical effect.

Developmental effects (decreased fetal weights) are considered the critical effect and serve to identify the sensitive population—the fetus of the pregnant female. The studies by Price et al. (1996; 1994), Heindel et al. (1992), and NTP (1990) in rats were chosen as critical developmental studies because they were well-conducted studies of a sensitive endpoint that identified both a NOAEL and LOAEL. Rats were more sensitive than mice and rabbits, which were also studied for developmental toxicity.

The POD was determined by BMD modeling. BMD evaluation of multiple developmental endpoints identified decreased fetal body weight as the most suitable endpoint. Two studies (Price et al., 1996; Price et al., 1994; Heindel et al., 1992) provided data on fetal body weight, and the results were combined for BMD evaluation. The benchmark response (BMR) level for mean fetal weight was chosen to be the $BMDL_{05}$ value of 10.3 mg/kg-day.

No data are available to identify an MOA, but boron is absorbed, distributed, and eliminated unchanged in urine. It is not metabolized, so some measure of exposure to the parent compound should serve as the basis for dose (exposure) expression.

A.3.1.3. *Basis for Data-Derived Extrapolation Factor*

Following administration, boron is rapidly absorbed and distributed throughout the body. It distributes with total body water; concentrations in all tissues examined were similar. Bone and fat tissues represent outliers—boron seems to accumulate in bone and the low water content of adipose tissue reduces boron distribution to fat.

Given the relatively uniform distribution of boron to the tissues and that the majority of the compound is excreted quickly, the likelihood for sequestration of boron by a given tissue is minimal. Although there are no direct measurements of fetal boron concentrations, boron concentrations in the fetus should be the same as in the mother because boron is freely diffusible across biological membranes and will rapidly and evenly equilibrate in all body water compartments. As the boron RfD is based on developmental effects observed in rats, the most relevant kinetic data are those pertaining to pregnant rats and pregnant humans. Given the difficulty in obtaining tissue boron concentrations in the developing fetus, data on plasma boron in these species were considered; however, data were insufficient to compare plasma boron in rats and humans at the same exposure levels. Therefore, boron clearance is used as an estimator of internal dose. Again, complications of the availability of data on boron clearance in a large enough population sufficient to support reliable estimates of variability were identified.

Since boron is not metabolized, clearance from blood and tissues is via urinary elimination. Boron is a small, uncharged molecule, and data indicate a lack of protein binding. Evidence from human dialysis studies indicates clearance is via passive diffusion. These (and other) data identify passive renal mechanisms as those most governing boron clearance. Because

the molecular and physical attributes of boron were consistent with those for agents eliminated by glomerular filtration and because boron clearance correlated with measures of glomerular filtration in some studies, variability of GFR was deemed an acceptable surrogate for variability of boron clearance among pregnant women. Table A-4 lists several studies that have characterized the variability of GFR among pregnant humans. The application of these data describing variance of GFR among pregnant humans serves as the basis for estimating human intraspecies differences in internal exposure.

Table A-4. Measures of glomerular filtration rate variability among pregnant women

Study	Mean GFR (mL/min)	Standard Deviation
Dunlop	150.5[a]	17.6
Krutzen	195[b]	32
Sturgiss	138.9[c]	26.1

[a]Serially averaged observations across three time periods (16, 26, and 36 weeks) for 25 pregnant women.

[b]Third-trimester values for 13 pregnant women.

[c]Serially averaged observations across two time periods (early and late pregnancy) for 21 pregnant women (basal index plus basal control individuals).

A.3.1.4. *Data-Derived Extrapolation Factor Derivation*

For the assessment of intraspecies TK variability, GFR is used as a surrogate for boron clearance. Although the study of Pahl et al. (2001) provides an estimate of boron clearance variability in pregnant women, the data are judged to be inadequate for this purpose. As boron clearance is largely a function of GFR, GFR is considered to be an appropriate surrogate and there is a larger, more certain database on GFR and its variability among humans than on boron clearance. Thus, the GFR database is used to estimate boron clearance variability. Because the measured boron clearances in the rat and human kinetic studies were less than GFR, tubular reabsorption could be contributing to the variability of boron clearance. Variability in these factors, however, is judged to be minor in comparison to the variability in GFR.

GFR data have been used previously in the context of the boron RfD by Dourson et al. (1998), who proposed the ratio of the mean GFR to the GFR value two standard deviations (SDs) below the general population mean (mean ÷ [mean − 2 SD]) as the metric for the EF_{HK}. This

approach is referred to as the sigma method, which is a common term used for statistical methods using multiple SDs to establish "acceptable" lower bounds.

For the derivation of EF_{HK}, for reasons described here, the sigma method is modified by using 3 SD as the reduction factor for establishing the lower bound (i.e., mean GFR − 3 SD). The basic formula modified from Dourson et al. (1998) for EF_{HK} is:

$$GFR_{AVG}^{EFHK} = GFR_{AVG} - 3\ SD_{GFR} \qquad \text{(A-5)}$$

where GFR_{AVG} and SD_{GFR} are the mean and SD of the GFR (mL/minute) for the general healthy population of pregnant women. The use of 3 SD rather than 2 SD (as in Dourson et al., 1998) is based on a statistical analysis of the published GFR data, with more consideration being given to the full range of GFR values likely to be found in the population of pregnant women. In the aggregate, the data suggest that a lower bound GFR 2 SD below the mean does not provide adequate coverage of the susceptible subpopulation (those pregnant women experiencing or predisposed to preeclampsia who have lower GFR values). While no conclusive information exists from controlled-dose studies in humans, it may be possible that the variability in boron clearance might be greater than GFR variability, but this is not expected. The uncertainty surrounding this possibility is low. Therefore, EF_{HK} must also account for any residual uncertainty in using GFR as a surrogate.

The three studies listed in Table A-4 (Sturgiss et al., 1996; Krutzén et al., 1992; Dunlop, 1981) were found to address GFR variability in pregnant women. Dunlop (1981) assessed GFR for 25 women at 3 different time points during pregnancy (16, 26, and 36 weeks) and again after delivery. In this study, GFR was measured as inulin clearance and the overall average and SD was 150.5 and 17.6 mL/minute, respectively. Sturgiss et al. (1996) performed a similar assessment of GFR (also using inulin clearance) for 21 women in early (12−19 weeks) and late (30−35 weeks) pregnancy and again at 15−25 weeks postpartum and found a mean GFR of 138.9 mL/minute with an SD of 26.1 mL/minute. Krutzén et al. (1992) evaluated GFR during pregnancy for 4 different groups of women (13 normal healthy women, 16 diabetic women, 8 hypertensive women, and 12 women diagnosed with preeclampsia) by using iohexol clearance in the second and third trimester and again 6−12 months postpartum. Krutzén et al. (1992) reported the third trimester mean GFR and SD for the healthy women as 195 and 32 mL/minute, respectively. In general, the GFR values reported in this study are much higher than those reported by Sturgiss et al. (1996) and Dunlop (1981). The reason for this discrepancy is not known. The GFRs from these studies and the results of the sigma method value calculations for EF_{HK} are shown in Table A-5.

Table A-5. Sigma-method value calculation for intraspecies toxicokinetic extrapolation factor[a]

Study	Mean GFR (SD) (mL/min)	Mean GFR − (3 SD)	Sigma-Method Value
Dunlop	150.5 (17.6)[b]	97.7	1.54
Krutzen	195 (32)[c]	99	1.97
Sturgiss	138.9 (26.1)[d]	60.6	2.29
Averages	161.5	85.8	1.93

[a]Mean GFR ÷ (Mean GFR − 3 SD).

[b]Serially averaged observations across 3 time periods (16, 26, and 36 weeks) for 25 pregnant women.

[c]Third-trimester values for 13 pregnant women.

[d]Serially averaged observations across two time periods (early and late pregnancy) for 21 pregnant women (basal index plus basal control individuals).

Considering the Krutzén et al. (1992) results in the context of the sigma method, a reduction of 2 SD from the healthy population mean to establish the lower bound (which results in a GFR slightly higher than the mean of the preeclamptic GFR) would appear to be insufficient for adequate coverage of the susceptible population. Thus, the use of 3 SD below the healthy GFR mean gives coverage in the sensitive subpopulation to about 1 SD below the mean preeclamptic GFR.

As no single study is considered to be definitive for assessment of population GFR variability, EF_{HK} is determined from the average of the individual sigma-method values for each of the three studies. The mean GFR and SD values in Table A-5 are based on average GFR across the entire gestational period, except for the Krutzén et al. (1992) estimate, which was for the third trimester only. The average sigma-method value from the three studies is 1.93. Considering a small residual uncertainty in the use of GFR as a surrogate for boron clearance, the average sigma-method value of 1.93 is rounded upward to 2.0 and established as the value for EF_{HK}.

By virtue of their lower GFR, pregnant women diagnosed with preeclampsia are considered to be a sensitive subpopulation, at least toxicokinetically. TD sensitivity is presumably independent of TK sensitivity. The onset of preeclampsia generally occurs after week 20 of pregnancy and is characterized by acute hypertension, often accompanied by edema and proteinuria. Women with preeclampsia are at increased risk for premature separation of the placenta from the uterus and acute renal failure, among other adverse health effects. The fetus may become hypoxic and is at increased risk of low birth weight or perinatal death.

The approximately twofold intraspecies variability factor derived from 3 SDs below the mean of three studies for pregnancy GFR (mean = 161.5 mL/minute; mean − 3 SD = 85.8) is considered preferable for providing adequate coverage to women predisposed to adverse birth outcomes due to renal complications. Therefore, the default value of 3.16 for intraspecies UF for the TK component (UF_{HK}) was obviated by a DDEF of 2.0.

A.3.1.5. *References for Case Study A.3.1*

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A.3.2. Methylmercury—Intraspecies Extrapolation Factor for Toxicokinetics Case Study

A.3.2.1. *Summary*

This case study presents information derived from the *Water Quality Criterion for the Protection of Human Health: Methylmercury* (U.S. EPA, 2001d) and the IRIS entry for methylmercury (MeHg) (U.S. EPA, 2001b). MeHg has an RfD of 1×10^{-4} mg/kg-day in EPA's IRIS database (U.S. EPA, 2001b). No Toxicological Review is available for MeHg, but the underlying data and interpretations are published in the *Water Quality Criterion for the Protection of Human Health: Methylmercury* (U.S. EPA, 2001d).

Multiple RfDs were calculated from $BMDL_{05}$ values for various endpoints reported in three epidemiological studies measuring neurobehavioral deficits in children exposed *in utero*. Composite UFs of 10 were used in all calculations. This included a default threefold factor for human TD variability and uncertainty and a threefold factor for human TK variability and uncertainty. This latter EF_{HK} was based on published analyses of human TK data.

A.3.2.2. *Hazard Identification and Dose-Response*

MeHg can produce a variety of toxicities depending on the dose. These range from seizures and death to subtle neurobehavioral changes in humans exposed *in utero*. The choices of studies, critical effects, model, and POD were informed by an NRC advisory report and a subsequent review by an independent scientific panel (U.S. EPA, 2000b).

Mercury is methylated in soils and sediments by microorganisms and is bioaccumulated through aquatic food webs. It can reach relatively high concentrations (1 ppm or more) in predatory fish and sea mammals consumed by humans and wildlife. MeHg is absorbed readily from the human gut and is transported through the body, crossing both the blood/brain and placental barriers. Human studies from environmental exposures (fish and seafood consumption) were available and served to define the POD. Neurobehavioral effects were observed in two studies of children exposed *in utero* from maternal consumption of seafood [Faroe Islands and New Zealand; (U.S. EPA, 2001d)]. The Faroe Islands study was a longitudinal study of about 900 mother-infant pairs (Grandjean et al., 1997). The main independent variable was cord-blood mercury; maternal hair mercury was also measured as was child hair mercury. At 7 years of age, children were tested on a variety of tasks designed to assess function in specific behavioral domains. In the New Zealand study (Kjellstrom et al., 1989; Kjellstrom et al., 1986), increased maternal hair mercury was associated with decreased scores on standard intelligence quotient tests in 6-year-old children. No effects were reported in a third such study in the Seychelles Islands, but these data were also included in the modeling (NRC, 2000).

No MOA for MeHg has been established.

Test responses of children in three large studies discussed above (Faroe Islands, Seychelles, and New Zealand) were coupled with measured or calculated MeHg cord-blood concentrations for the dose-response analysis. BMD analysis was applied to the results from multiple individual neurobehavioral tests (e.g., Boston Naming Test, Continuous Performance Test, and California Verbal Learning Test). Data were modeled using a *K*-power model with $K \geq 1$; $K = 1$ generally giving the best fit. These data were continuous in exposure and effect. An abnormal response was defined as one falling into the lowest 5% of test responses ($P_0 = 0.05$). The BMR was set at 0.05, based on the NRC committee's advice that the combination of BMR and P_0 were within the observed range of responses and were, in fact, typical for these types of measurements (NRC, 2000). $BMDL_{05}$ values of 46 to 79 ppb MeHg in fetal cord blood were chosen as the points of departure for RfD calculation.

There is a correlation between maternal-blood mercury concentrations and fetal-blood mercury concentrations. A review of results from 21 studies demonstrated that the ratio of concentrations (fetal:maternal) is typically higher than 1, with overall mean values supporting a ratio close to 1.7. Based on the advice of an NRC panel, EPA (U.S. EPA, 2001b) chose not to make a numerical adjustment between cord-blood and maternal-blood mercury in calculating the RfD. The relationship between cord-blood and maternal-blood mercury was instead discussed as an area of variability and uncertainty during UF derivation.

Twenty-four RfDs were calculated using various $BMDL_{05}$ values but with the same dose conversion and a composite UF of 10. These calculations resulted in one RfD = 0.2 μg/kg bw/day, three RfD = 0.05 μg/kg bw/day, and twenty RfD = 0.1 μg/kg bw/day (or 1×10^{-4} mg/kg-day).

A.3.2.3. *Basis for Data-Derived Extrapolation Factor*

A PBPK model and a one-compartment model for pregnant women were used to examine the relationship between ingested doses of MeHg and maternal blood levels. To estimate human intraspecies variability (of MeHg concentrations in maternal blood to ingested MeHg dose), the most deterministic (sensitive) parameters of the TK model were identified and varied. Model results demonstrated that external doses required to produce maternal-blood concentrations of 1 ppm varied up to threefold. This value (3) served as a nondefault value for EF_{HK}; the TD component of the intraspecies UF (UF_{HD}) was left at a default value of 3 and the overall intraspecies UF value was 10.

A.3.2.4. *Data-Derived Extrapolation Factor Derivation*

Multiple measures of MeHg exposure are available from several human studies. For MeHg, hair and blood are considered more appropriate than urine, particularly for longer term exposure. The toxicity evaluated was induced during gestation, at a time when MeHg exposure to the developing brain of the fetus is through the placental blood supply. Thus, MeHg originated from the maternal blood circulation. Both a PBPK model and a one-compartment model were used to assess variables in fetal MeHg exposure. Independent of model type, ability to estimate maternal hair concentrations required at least two more parameters (blood-to-hair transfer and hair growth rate) than was required to estimate maternal blood concentrations. In addition, because EPA set cord-blood concentrations to equal maternal-blood concentrations, the model was able to predict fetal cord blood concentrations with less uncertainty than maternal hair concentrations. Largely for these reasons, blood MeHg concentrations were selected as the most appropriate dose metric.

EPA characterized human TK variability as differences in external (ingested) doses of MeHg that resulted in the same concentration of MeHg in maternal blood. The concentration selected for analysis was one that was relevant to the $BMDL_{05}$ for the neurobehavioral effects—namely 1 ppm. This concentration is about 12 to 20 times higher than the concentrations serving as the POD, but the choice was based in part on increasing model uncertainty when predicting concentrations lower than 1 ppm. An evaluation of the uncertainty and variability in model parameters was conducted in three studies (Swartout and Rice, 2000; Clewell et al., 1999; Stern, 1997) to identify the extent to which the external (ingested) dose might vary when compared to a fixed maternal hair or blood concentration.

Results from the Stern (1997) analysis were available in the original publication, whereas specific predictions of values at given percentiles for the Swartout and Rice (2000) and Clewell et al. (1999) studies required additional model exercises by the original authors; these analyses were published by NRC (2000). All data used in the models were from human studies. The analysis demonstrated the ratio of external (ingested) doses (in µg/kg-day) that resulted in the same blood concentration. The value of 3 was selected to represent the TK portion of the intraspecies extrapolation. This value was at or above the estimates from all three analyses for the comparison of the dose at the 50th percentile of the distribution to the dose at the 1st percentile of the distribution. The selected value of 3 thus encompassed the difference across these percentiles of the distribution. Because the dose at the 1st percentile of the distribution is lower than the dose at the 50th percentile of the distribution, the ratio has a value greater than 1.0.

Table A-6 presents the ratios developed (external dose at the 50th percentile/external dose at the 1st percentile) for each of the three studies. Considering TK variability as described

by the ratio of external doses at the specified percentiles of the distribution, values for blood and hair ranged from 1.7 to 3.3. Maximum values were 3.3 and 3.0 for hair and blood, respectively. EPA's IRIS entry for MeHg states, "Using maternal blood as the starting point, the consolidated range from the three analyses is 1.7 to 3.0." This is consistent with EPA's Water Quality Criterion document (U.S. EPA, 2001d). On this basis, a value of 3 was chosen to represent the TK portion of intraspecies variability. As no data were available to address intraspecies differences in susceptibility (TD), this portion of the UF was left at the default value of 3. Together these values for the components of UF_H combine to equal a value of 10, which was characterized as a "hybrid" value, comprising values based on default methodology and on data.

Table A-6. Comparison of results from three analyses of the intraspecies variability in the ingested dose of methylmercury corresponding to a given maternal-hair or blood mercury concentration

Study	Maternal medium	50th Percentile[a] (µg/kg-day)	50th Percentile/ 5th percentile[b]	50th Percentile/ 1st percentile[c]
Stern (1997)	Hair	0.03−0.05[d] (mean = 0.04)	1.8−2.4 (mean = 2.1)	2.3−3.3 (mean = 2.7)
	Blood	0.01	1.5−2.2 (mean = 1.8)	1.7−3.0 (mean = 2.4)
Swartout and Rice (2000)	Hair	0.08	2.2	Data not reported
	Blood[e]	0.02	2.1	2.8
Clewell et al. (1999)	Hair	0.08	1.5	1.8
	Blood[f]	0.07	1.4	1.7

[a]Predicted 50th percentile of the ingested dose of MeHg that corresponds to 1 ppm Hg in hair or 1 ppb in blood.
[b]Ratio of 50th percentile of ingested dose of MeHg that corresponds to 1 ppm Hg in hair or 1 ppb in blood to the 5th percentile.
[c]Ratio of 50th percentile of ingested dose of MeHg that corresponds to 1 ppm Hg in hair or 1 ppb in blood to the 1st percentile.
[d]Range reflects minimum and maximum values among eight alternative analyses.
[e]Data from J. Swartout, U.S. EPA, personal communication; June 9, 2000.
[f]Data from H.J. Clewell, ICF Consulting, personal communication; April 19, 2000 as cited in NRC (2000).

SOURCE: This is Table 3-1 from NRC (2000).

Acknowledgment: Some passages in this document were taken from EPA's IRIS entry for Methylmercury (U.S. EPA, 2001b); some were taken from EPA's Water Quality Criterion document for Methylmercury (U.S. EPA, 2001d).

A.3.2.5. *References for Case Study A.3.2*

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