Harmonization Project Document No. 2

CHEMICAL-SPECIFIC ADJUSTMENT FACTORS FOR INTERSPECIES DIFFERENCES AND HUMAN VARIABILITY: GUIDANCE DOCUMENT FOR USE OF DATA IN DOSE/CONCENTRATION–RESPONSE ASSESSMENT

This project was conducted within the IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals.

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World Health Organization
Geneva, 2005

The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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TABLE OF CONTENTS

FOREWORD

Harmonization Project Documents are a new family of publications from the International Programme on Chemical Safety (IPCS) — a cooperative programme of the World Health Organization (WHO), the International Labour Organization (ILO) and the United Nations Environment Programme (UNEP). Harmonization Project Documents join the Environmental Health Criteria (EHC) methodology (yellow cover) series of documents as authoritative documents on methods for the risk assessment of chemicals.

The main impetus for the current coordinated international, regional and national efforts on the assessment and management of hazardous chemicals arose from the United Nations Conference on Environment and Development (UNCED) held in 1992 and was reconfirmed at the 2002 World Summit on Sustainable Development. UNCED Agenda 21, Chapter 19, the "blueprint" for the environmentally sound management of toxic chemicals under the principles of sustainable development, has guided most international and national chemical-related activities. Chapter 19 is the agreed upon, endorsed international programme of action of governments for developing and implementing national programmes for management of chemicals within the principles of sustainable development.

The IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (Harmonization Project) is conducted under Agenda 21, Chapter 19. The Intergovernmental Forum on Chemical Safety (IFCS) Forum III, held in Salvador da Bahia in October 2000, agreed on Priorities for Action Beyond 2000, which further define the actions recommended to be taken. Forum III declared that by 2004, IPCS and the Inter-Organization Programme for the Sound Management of Chemicals (IOMC, which comprises seven intergovernmental organizations) should have ensured that recommendations for harmonized assessment approaches were available for terminology, cancer and reproductive and developmental toxicology and that common principles for the assessment approach to other specific toxicological end-points, such as immunotoxicology, endocrine disruptors and ecotoxicology, should be adopted wherever possible.

The IPCS Harmonization Project, which is ongoing, states that "harmonization," in the context of chemical risk assessment, should not simply be equated with standardization. It is not a goal of the project to standardize risk assessments globally, as that is considered to be neither appropriate nor feasible. Instead, harmonization is thought of as an effort to strive for consistency among approaches and to enhance understanding of the various approaches to chemical risk worldwide. Thus, harmonization is defined, in a step-wise fashion, as an understanding of the methods and practices used by various countries and organizations so as to develop confidence in, and acceptance of, assessments that use different approaches. It further involves a willingness to work towards convergence of these approaches or methods as a longer-term goal.

Achieving harmonization of approaches is considered to provide a framework for comparing information on risk assessment; understanding of the basis for exposure standards for specific chemicals in different countries; savings of time and expense by sharing information and avoiding duplication of work; and credible science through better communication among

organizations and peer review of assessments and assessment procedures. The stated project mission is to ensure better chemical risk assessment and hence management practices that promote the protection of human health and the environment within the framework of sustainable development.

This ongoing project is overseen by a geographically representative Harmonization Project Steering Committee and a number of ad hoc Working Groups that manage the detailed work. Finalization of documents includes a rigorous process of international peer review and public comment.

PREFACE

This guidance document was prepared under the auspices of the IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals. The Working Group that planned and initiated the work comprised the following members:

Erik Dybing, National Institute of Public Health, Norway (Chair)
Robin Fielder, Department of Health, United Kingdom
Donald Grant, Consultant to IPCS, Canada
Ursula Gundert-Remy, Federal Institute for Health Protection of Consumers and Veterinary Medicine (BGVV), Germany
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Maged Younes, Secretariat, IPCS

The draft guidance document was further developed with input from an IPCS Workshop on Incorporating Uncertainty and Variability into Risk Assessment, held in May 2000 in Berlin, Germany, and through a follow-up meeting convened in August 2000 in Ottawa, Ontario, Canada, by the following Drafting Group, consisting of several of the Working Group members and participants in the Berlin Workshop:

Alan Boobis, Imperial College School of Medicine and Technology, United Kingdom
Michael Dourson, Toxicology Excellence for Risk Assessment, USA
Donald Grant, Consultant to IPCS, Canada
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The draft guidance document was circulated for review by all participants in the Berlin Workshop, review comments were incorporated and the guidance document was finalized for peer review in 2001.

The document was placed on the IPCS Internet site for open public comment/peer review in late 2001. However, no comments were received. In late 2003, it was decided to call for

another round of review, and the document was distributed to relevant WHO Collaborating Centres, IPCS Participating Institutions and selected experts involved in IPCS activities. Twelve submissions containing comments were received. Dr Andrew Renwick prepared a revised document, taking into account the comments received, for consideration by a newly constituted expert group comprising some members of the Drafting Group listed above and selected reviewers from the peer review round. The group members were:

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LIST OF ACRONYMS AND ABBREVIATIONS

AD_{AF}	chemical-specific adjustment factor for interspecies differences in toxicodynamics
AD_{UF}	default uncertainty factor for interspecies differences in toxicodynamics
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AK_{AF}	chemical-specific adjustment factor for interspecies differences in toxicokinetics
AK_{UF}	default uncertainty factor for interspecies differences in toxicokinetics
AUC	area under the concentration–time curve
BMC	benchmark concentration
BMC_{05}	the concentration (or its lower confidence limit) calculated to be associated with a 5% incidence of effect
BMD	benchmark dose
C_{max}	maximum concentration delivered to target organ
CL	clearance
CSAF	chemical-specific adjustment factor
CUF	composite uncertainty factor
CYP	cytochrome P450
EC_{10}	10% effective concentration
EHC	Environmental Health Criteria monograph
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
GSD	geometric standard deviation
HD_{AF}	chemical-specific adjustment factor for human variability in toxicodynamics
HD_{UF}	default uncertainty factor for human variability in toxicodynamics
HK_{AF}	chemical-specific adjustment factor for human variability in toxicokinetics
HK_{UF}	default uncertainty factor for human variability in toxicokinetics
IFCS	Intergovernmental Forum on Chemical Safety
ILO	International Labour Organization
IOMC	Inter-Organization Programme for the Sound Management of Chemicals
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Committee on Food Additives and Contaminants
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K_m	Michaelis-Menten constant
LCL	lower confidence limit
LOAEC	lowest-observed-adverse-effect concentration
LOAEL	lowest-observed-adverse-effect level
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NOAEC	no-observed-adverse-effect concentration

NOAEL	no-observed-adverse-effect level
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
RfC	reference concentration
RfD	reference dose
SD	standard deviation
SEM	standard error of the mean
TDI	tolerable daily intake
UCL	upper confidence limit
UNCED	United Nations Conference on Environment and Development
UNEP	United Nations Environment Programme
V_{max}	maximum rate of metabolism
WHO	World Health Organization

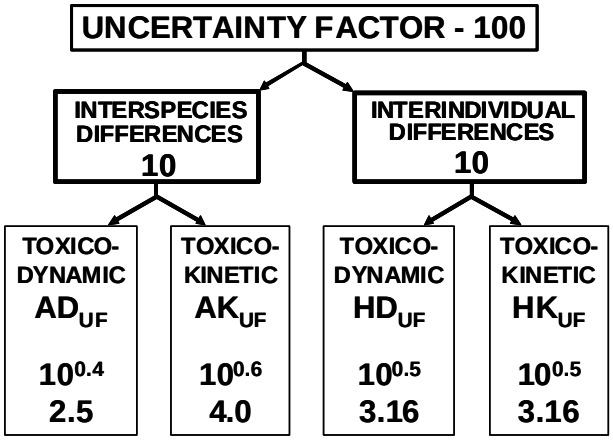
EXECUTIVE SUMMARY

Default safety/uncertainty factors have been used for over 40 years to estimate health-based guidance values based on no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) from studies in animals. A value of 100 is normally used by bodies such as the Joint FAO/WHO Committee on Food Additives and Contaminants (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) to derive an acceptable daily intake (ADI), a tolerable daily intake (TDI) or a reference dose (RfD) for the general population based on a NOAEL or LOAEL from a chronic study in animals. This value represents the product of two factors of 10, which allow for interspecies differences and human variability. Extra uncertainty factors, which are not part of the general uncertainty factor used to allow for interspecies differences or human variability, are sometimes used to allow for database deficiencies and for the severity and irreversibility of effects. These are not considered further in this guidance document.

The concept of chemical-specific adjustment factors (CSAFs) has been introduced to provide a method for the incorporation of quantitative data on interspecies differences or human variability in either toxicokinetics or toxicodynamics (mode of action) into the risk assessment procedure, by modifying the relevant default uncertainty factor of 10. Incorporation of toxicokinetic or toxicodynamic data becomes possible if each factor of 10 is divided into appropriately weighted subfactors. The contribution of toxicokinetics and toxicodynamics to each factor of 10 (i.e., the appropriate weighting of subfactors) was discussed at an International Programme on Chemical Safety (IPCS) meeting in 1994. As a result of these discussions, default values were derived for subfactors, which, when multiplied, give the original default values of 10 (see Figure S-1). The split between toxicokinetics and toxicodynamics may be different in other situations, but the overall approach described in this document would still be appropriate.

When appropriate chemical-specific data are available, a CSAF can be used to replace the relevant default subfactor; for example, suitable data defining the difference in target organ exposure in animals and humans could be used to derive a CSAF to replace the AK_{UF} subfactor for animal to human differences in toxicokinetics (4.0 in Figure S-1). The total uncertainty factor (the composite uncertainty factor, or CUF) that would be used in the risk assessment would be the composite value obtained on multiplying the CSAF, used to replace a default subfactor, by the remaining default subfactors for which suitable data were not available. In this way, chemical-specific data in one area could be introduced quantitatively into the derivation of a health-based guidance value, such as an ADI, TDI or RfD, and data would replace uncertainty. The approach under which CSAFs would be used in risk assessment has been such that in the absence of data, the usual default uncertainty factor would be used. This does not necessarily mean that the default of 100 is the ideal value; it is simply recognition that this reflects the common current approach to deriving a health-based guidance value for the general population.

This guidance document describes the types and quality of data that could be used to derive a CSAF. The guidance is separated into four main sections covering each of the four different



AD_{UF} = Uncertainty factor for animal to human differences in toxicodynamics
AK_{UF} = Uncertainty factor for animal to human differences in toxicokinetics
HD_{UF} = Uncertainty factor for human variability in toxicodynamics
HK_{UF} = Uncertainty factor for human variability in toxicokinetics

Figure S-1. Subdivision of the usual uncertainty factor of 100 used in setting guidance values for the exposure of the general population, such as ADIs, TDIs or RfDs. Different numerical values could be derived if the usual total default uncertainty factor were not 100 — for example, in the risk assessment of occupational exposures (based on IPCS, 1994).

areas where CSAFs can be introduced to replace a default subfactor (shown as the bottom row in Figure S-1) plus a section describing how the CUF is determined:

- data related to interspecies differences in toxicokinetics
- data related to interspecies differences in toxicodynamics
- data related to human variability in toxicokinetics
- data related to human variability in toxicodynamics
- combination of adjustment factors and default uncertainty factors to derive a CUF.

The text for each section is self-contained, so that risk assessors can determine the adequacy of chemical-specific data related to any one of the possible default subfactors without reference to the other subfactors that were not under consideration due to the absence of any relevant data. Because of this, there is intentional repetition in section 3.

The production of chemical toxicity represents a continuum of processes usually involving uptake from the site of administration, delivery to the target tissue/organ, uptake from the circulation by the target tissue/organ and responses within the target tissue/organ. Each factor of 10 in Figure S-1 allows for all aspects of the overall process, but the introduction of chemical-specific data related to either toxicokinetics or toxicodynamics requires that the default factor be split into two appropriately weighted subfactors. Bioactivation and detoxication processes that occur within the target tissue/organ cannot normally be assessed directly from circulating concentrations of the parent compound or its metabolites, but measurements

of the concentrations of the parent compound or its metabolites in the general circulation will reflect major sources of interspecies differences and human variability in tissue/organ delivery.

For application of CSAFs, the continuum of processes leading to chemical toxicity was split at the level of delivery of the parent compound or a circulating active metabolite to the target tissue/organ: events up to this point were considered as toxicokinetics, and events within the target tissue/organ were considered as toxicodynamics. The reason for this subdivision between kinetics and dynamics was that the data used to subdivide the factors for interspecies differences and human variability (the two factors of 10 in Figure S-1) into toxicokinetic and toxicodynamic aspects were derived largely from physiological differences between rodents and humans for interspecies differences and from the clinical pharmacology literature for human variability, based on plasma concentration measurements (toxicokinetics) and data from *in vitro* studies or from modelling of data from *in vivo* studies in humans (toxicodynamics). In consequence, the data used to replace a default subfactor for toxicokinetics will usually be based on the concentrations of the chemical or active metabolite in the general circulation. Physiologically based pharmacokinetic (PBPK) models can be used to develop CSAFs; reconsideration of the subdivision between kinetics and dynamics would be necessary if the PBPK model incorporated bioactivation and/or detoxication processes within the target tissue/organ. Similar reconsideration would be necessary if the model related to an effect at the site of contact.

The interspecies default subfactors for toxicokinetics or toxicodynamics could be replaced by data that adequately defined the difference in the mean parameter estimates between the test species in the study giving the NOAEL or LOAEL and adult humans. The choice of the appropriate parameter estimate for toxicokinetics or toxicodynamics would relate to the nature of exposure in the study and the critical effect. In some cases, data on physiological or biochemical processes may also be applicable in this framework. Adequate data would provide a reliable measurement of the central tendency for the parameter in the test species at the NOAEL or LOAEL and in humans at appropriate exposures.

The default subfactors for toxicokinetics or toxicodynamics for human variability could be replaced by data that defined the variability in the relevant parameter estimates in healthy human adults, including the influence of any functional genetic polymorphism, as well as the variations between different potentially susceptible subgroups as appropriate. The default subfactors for human variability are single values of $10^{0.5}$ or 3.16 (when based on a default uncertainty factor of 10), whereas the analysis of data on human variability will result in a distribution, such as a lognormal distribution, of an appropriate parameter to reflect kinetic or dynamic variability. Replacement of the default subfactor by a CSAF will require analysis of the distribution to give a point estimate related to a percentile of the distribution. The percentile that would be used would be a policy decision and could be influenced by aspects such as the severity of the effect, the robustness of the data, the nature of the distribution and risk management considerations. Examples of potentially suitable percentiles that might be provided to the risk manager are the 90th, 95th or 97.5th percentile; the CSAF would be calculated as the parameter estimate at the percentile of interest divided by the parameter estimate at the mean. Where there are discrete subgroups of the population, the CSAFs for

different percentiles should be calculated based on data for the whole population, including the subgroup, and also for the subgroup separately. Both sets of results should be provided to the risk manager. Consideration of population distributions as a part of risk assessment should facilitate future developments on the use of probabilistic approaches.

The numerical value for a CSAF is that dictated by the data and could range from less than 1 to considerably more than the default subfactor; in consequence, the CUF may be either less than or more than the usual default, typically 100. If the CUF is less than the usual default value (e.g., 100) for a particular end-point or adverse effect, then it is necessary to consider if other end-points to which the usual default value would be applied might become the toxic effect of concern that determines the risk assessment outcome.

Although suitable data may be available only rarely, analysis of available data on a chemical using the framework presented in this guidance document provides a useful method of assessing the overall adequacy of the data for risk assessment purposes. In addition, application of the principles described can assist in the identification and filling of data gaps that would help to improve the risk assessment.

1. INTRODUCTION

The objective of this document is to provide guidance to risk assessors on the use of quantitative toxicokinetic and toxicodynamic data to address interspecies and interindividual differences in dose/concentration–response assessment. Section 1 focuses on the relevance of this guidance in the context of the broader risk assessment paradigm and other initiatives of the International Programme on Chemical Safety (IPCS) project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (Harmonization Project). Technical background material is presented in section 2, followed by generic guidance for the development of chemical-specific adjustment factors (CSAFs) in section 3 and accompanying summary figures. Illustrative case-studies are included in Appendix 1, and a glossary of terms is provided in Appendix 2.

1.1 Objectives

The principal objectives of this guidance document are 1) to increase common understanding and to encourage the incorporation of relevant quantitative data in a context consistent with traditional approaches to development of measures of dose/concentration–response and 2) to more fully delineate appropriate avenues of research to enable more predictive estimates of risk. With respect to the latter objective, this approach necessarily requires ethically derived human data from either *in vivo* or *in vitro* studies in order to inform the selection of appropriate adjustment factors for interspecies differences or human variability. The types of data considered to be informative in this context are also often from *in vitro* animal studies, consistent with objectives to reduce and/or replace the use of animals in toxicological testing. The approaches described in the following sections are also amenable to presentation in a probabilistic context (rather than development of single measures for dose/concentration–response), where data available are sufficient to meaningfully characterize the distribution of interest.

Thus, this guidance on the incorporation of quantitative data on interspecies differences or human variability in toxicokinetics and toxicodynamics into dose/concentration–response assessment through the development of CSAFs is designed primarily for risk assessors. However, it is also relevant to those who commission or design relevant studies for the purposes of refining dose/concentration–response relationships. Indeed, it is hoped that the guidance included herein will encourage the development of appropriate data and facilitate their incorporation in dose/concentration–response assessment for regulatory purposes.

It is recognized that for many substances, there are few data to serve as a basis for development of CSAFs. Indeed, currently, relevant data for consideration are often restricted to the component of uncertainty related to interspecies differences in toxicokinetics. While there are commonly fewer appropriate, relevant data at the present time to address the three other components considered here — namely, interspecies (animal to human) differences in toxicodynamics, interindividual (human) variability in toxicokinetics and interindividual (human) variability in toxicodynamics — it is anticipated that the availability of such information will increase with a better common understanding of its appropriate nature. Consideration of the data needs for risk assessment, as described below in sections 3.1 to 3.4,

can be informative even in the absence of suitable data, because it focuses attention on gaps in the available information that, if filled, would permit development of a more refined risk assessment.

This guidance complements outputs of other initiatives of the IPCS Harmonization Project, where, for example, a framework for the transparent presentation of weight of evidence for a cancer mode of action has been developed (IPCS, 1999a; Sonich-Mullin et al., 2001). The approach described herein also presupposes that data that contribute to quantitative characterization of interspecies differences and human variability in the development of CSAFs have been critically reviewed and considered in the context of criteria for weight of evidence, such as consistency, etc. Relevant data often emanate from studies additional to those recommended routinely for toxicity testing, and multidisciplinary review of this information is encouraged.

1.2 Chemical-specific adjustment factors and risk assessment

In relation to risk assessment, a major area of advance has been an increasingly common understanding of the concept of "mode of action" and its contrast with "mechanism of action." In this context, "mode of action" is essentially a description of the processes that may lead to induction of the relevant end-point of toxicity for which the weight of evidence supports plausibility, whereas "mechanism of action" implies a more detailed molecular description of causality. The weight of evidence on hazard and mode of action for the spectrum of various end-points is assessed critically in order to define appropriate end-points for and approaches to characterization of dose/concentration–response. It is this latter component of risk assessment (i.e., assessment of dose/concentration–response relationships) for which the development of CSAFs is relevant.

In general, dose/concentration–response assessment is often based on only two or three data points in the experimental range. Either the experimental data are assessed to determine a level without adverse effects (the no-observed-adverse-effect level or NOAEL) or a curve is modelled that best fits the central estimates of the relationship defined by these experimental points and confidence intervals are calculated. There are two distinct approaches for extrapolation of risks to humans, based on the data within the experimental range — those that assume a threshold and those for which it is assumed that there is no threshold.

Generally, for all effects with the exception of those induced by direct interaction of the compound or its metabolites with genetic material, it is assumed that there is a threshold exposure below which the probability of harm is negligible. A presumed "safe" level of exposure is developed by division of a NOAEL, lowest-observed-adverse-effect level (LOAEL) or benchmark dose or concentration (BMD or BMC), which is a model-derived estimate (or its lower confidence limit) of a particular incidence level (e.g., 5%), by uncertainty factors to address principally interspecies and interindividual variation (IPCS, 1987, 1994). Alternatively, the magnitude by which the NOAEL (or LOAEL) or BMD/BMC exceeds the estimated exposure (i.e., the "margin of safety" or "margin of exposure") is considered in light of various sources of uncertainty and variability.

For effects involving direct interaction with genetic material (i.e., some types of carcinogenicity and germ cell mutagenicity), it is generally assumed that there is a probability of harm at any level of exposure. At present, there is no clear consensus on appropriate methodology for dose/concentration–response assessment in this case. Options include 1) expression of dose/exposure–response as potency in or close to the experimental range, 2) estimation of risks in the low-dose range through linear extrapolation from an effective dose, 3) calculation of the margin of exposure and 4) advice that control measures should be introduced to reduce exposure to the maximum extent practicable (Younes et al., 1998).

Inter- and intraspecies considerations are an essential part of extrapolation of experimental data from animal studies to humans and have been described as follows (IPCS, 1999b):

- *Interspecies consideration:* Comparison of the data for animals with the average of data for healthy humans, which will normally be young adults. Species differences result from metabolic, functional and structural variations.
- *Intraspecies or interindividual consideration:* Comparison of the representative healthy human with the range of variability present within the human population in relation to the relevant parameters, such as clearance (CL) or the 10% effective concentration (EC_{10}) (see sections 3.2 and 3.4).

It is the use of quantitative toxicokinetic and toxicodynamic data to inform interspecies and interindividual extrapolations in dose/concentration–response assessment (i.e., CSAFs) that is the focus of this guidance document. Previously, CSAFs were called "data-derived uncertainty factors" (Renwick, 1993; IPCS, 1994, 1999b). The new nomenclature of "chemical-specific adjustment factors" has been adopted because it better describes the nature of the refinement to the usual default approach. Also, it avoids confusion with factors that are based on an analysis of data for a group of chemicals sharing a common characteristic — for example, "categorical" factors, such as those based on common physical/chemical characteristics, which have also been referred to as data-derived factors.

It should be recognized that CSAFs represent part of a broader continuum of increasingly data-informed approaches to account for interspecies differences and human variability, which range from default ("presumed protective") to more "biologically based predictive" (Figure 1). The approach along this continuum adopted for any single substance is necessarily determined principally by the availability of relevant data. The extent of data available is, in turn, often a function of the economic importance of the substance.

The development of CSAFs may not always be possible or even necessary. For example, if the margin between the no- or lowest-effect level or BMD/BMC and anticipated human exposure is very large, the generation of the more sophisticated data necessary to replace part of a default uncertainty factor would not warrant the necessary experimentation in animals and humans and the associated resource expenditure. However, where this margin is small, development of additional chemical-specific quantitative data may be justified to refine the dose–response analyses and scientific credibility of the outputs, such as acceptable daily intakes (ADIs), tolerable daily intakes (TDIs), reference doses (RfDs), margins of exposure or margins of safety.

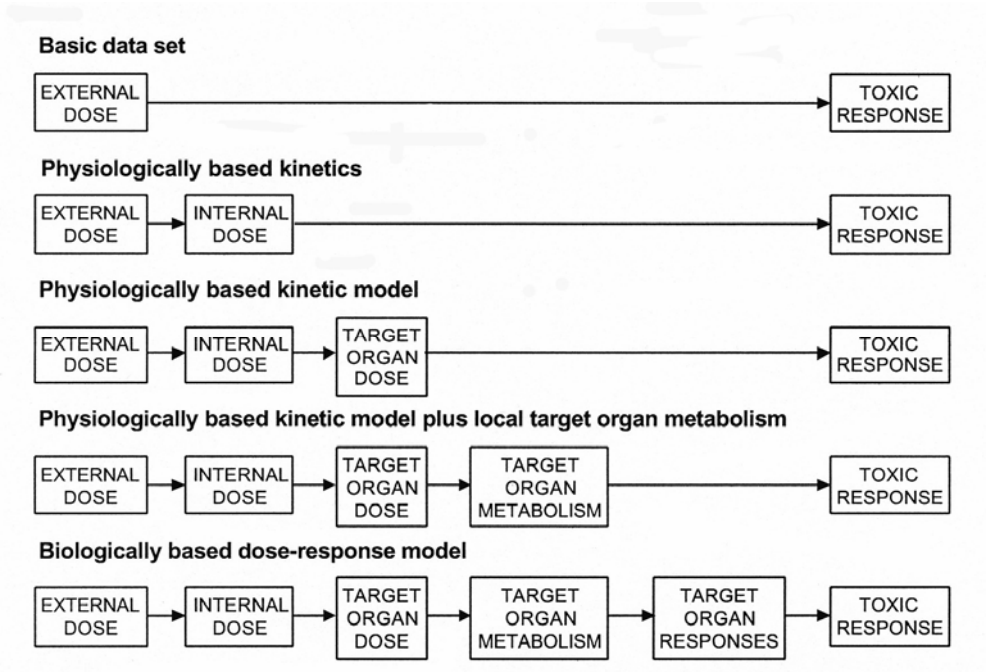


Figure 1. The relationship between external dose and toxic response for specific chemicals (from Renwick et al., 2001).

The focus of this guidance is the incorporation of quantitative data on toxicokinetics and toxicodynamics into dose/concentration–response analyses for approaches that lead to the estimation of a presumed "safe" (subthreshold) value, such as tolerable, acceptable or reference intakes or concentrations. However, it should be noted that the methodology described herein is equally applicable to the other approaches to exposure–response analyses, such as margins of exposure or linear extrapolation from estimates of potency close to the experimental range. It also lends itself to presentation of dose–response in a probabilistic context, where data are sufficient to confidently characterize the distributions of interest.

2. BACKGROUND

2.1 Framework for development of chemical-specific adjustment factors

The usual starting point for dose/concentration–response characterization of threshold effects is the NOAEL or BMD on a body weight basis (e.g., mg/kg body weight) or the no-observed-adverse-effect concentration (NOAEC) or BMC (i.e., concentration in air in, for example, mg/m^3) for the critical effect in animal studies. The NOAEL or NOAEC is the highest level of exposure that causes no detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organism. BMCs or BMDs are levels that cause specified levels of response for critical effects. NOAELs/NOAECs and BMDs/BMCs are normally divided by safety or uncertainty factors to derive levels of human exposure that will be without significant adverse effects. This represents the most common, but not the only, approach to dose/concentration–response characterization (US EPA, 1994; Edler et al., 2002). For simplicity of presentation in the following text, the NOAEL/NOAEC is used as the intake to which the uncertainty factor is applied, but the concepts described are equally applicable to the BMC or BMD and to the LOAEL or lowest-observed-adverse-effect concentration (LOAEC) (although an additional uncertainty factor may then be applied because a NOAEL was not defined in the study).

Traditionally, in relation to exposure of the general population, the NOAEL/NOAEC for the critical effect in animals has often been divided by an uncertainty factor of 100. The normal uncertainty factor of 100 can be regarded as comprising the product of two factors of 10, one for interspecies differences and one for interindividual variability in humans (IPCS, 1987). The two factors of 10 are default values and are applied to the NOAEL/NOAEC for different adverse effects detected in different test species; the aim of the present guidance document is to allow these defaults to be modified by chemical-specific data.

Where uncertainty factors different from 100 are used in other regulatory settings, the concepts of dividing the overall factor into different components and using chemical-specific data to modify the subfactors, as described in this document, still apply.

The interspecies uncertainty factor can be considered to convert the NOAEL/NOAEC for animals (derived from a small group of relatively homogeneous test animals) into the NOAEL/NOAEC anticipated for an average representative healthy human. The uncertainty factor for human variability converts the NOAEL/NOAEC for the average human into a NOAEL/NOAEC for susceptible humans. Although adverse effect data in humans can be used directly without the need for an interspecies factor, the paucity of such data means that the vast majority of risk assessments are based on studies in experimental animals.

Extra uncertainty factors, which are not part of the general uncertainty factor used to allow for interspecies differences or human variability, may be incorporated to allow for database deficiencies and for the severity and irreversibility of effects. These have been considered previously (IPCS, 1994) and are not considered further in this guidance document, because CSAFs would usually be derived for data-rich chemicals where database deficiencies would

not arise, and because the application of some additional uncertainty factors is a matter of subjective judgement.

2.2 Development of default subfactors for toxicokinetic and toxicodynamic aspects

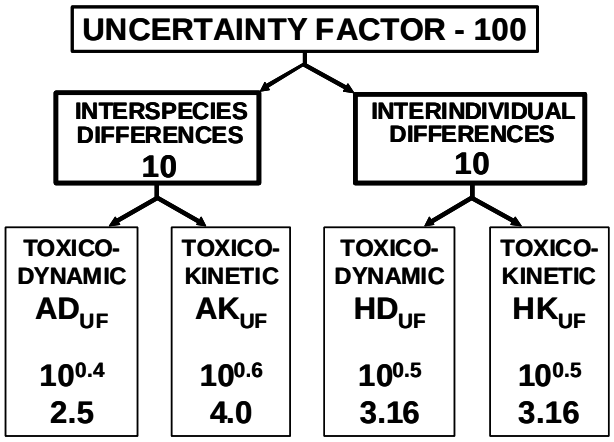
Procedures for characterization of dose/concentration–response currently range from the pragmatic approach of taking the experimental NOAEL/NOAEC and dividing by a commonly used default uncertainty factor (such as 100 for the general population based on animal data) to using a full biologically based model. In reality, the vast majority of databases upon which risk assessments are based include little information on either the delivery of the parent compound (or its circulating toxic metabolite) to the target tissue/organ or the mode of action. In consequence, the pragmatic default approach of using uncertainty factors has remained the cornerstone of characterization of dose/concentration–response.

Our increasing knowledge on interspecies differences and human variability in toxicokinetics, especially in foreign compound metabolism (Lipscomb & Kedderis, 2002), has emphasized the need for a method by which quantitative data can be incorporated into risk assessment. In order for either toxicokinetic data or data on mode or mechanism of action (i.e., toxicodynamic data) to contribute quantitatively to risk assessment, in the absence of a full biologically based dose–response or concentration–response model, it is necessary that the current procedure of applying factors of 10 for each of interspecies differences and human variability be refined. Subdivision of each factor of 10 into toxicokinetic and toxicodynamic components would allow part of the default value to be replaced by relevant, chemical-specific data when these were available, thereby advancing the scientific basis for dose–response or concentration–response characterization and improving confidence in the acceptable, tolerable or reference intakes or concentrations.

Human-derived data, most often obtained from *in vivo* studies in humans, are essential for consideration of either interspecies differences or human variability. Renwick (1993) analysed data on interspecies differences and human variability in toxicokinetics and toxicodynamics for a limited number of chemicals, the majority of which were pharmaceuticals administered orally or intravenously to human volunteers and patients. Subdivision of the uncertainty factors of 10 was based primarily on data for pharmacokinetic parameters, such as CL and area under the plasma or tissue concentration–time curve (AUC), because these relate directly to the steady-state body burden during chronic administration. The dynamics data for humans were based on *in vitro* dose–response data using tissues from humans or pharmacokinetic–pharmacodynamic modelling of a range of *in vivo* pharmacological and therapeutic responses, in which the interindividual variability in response is corrected for any interindividual variability in kinetics by the application of the model to the individual data. Based on available data, it was proposed that each of the factors of 10 could be subdivided into a factor of $10^{0.6}$ (4.0) for toxicokinetics and $10^{0.4}$ (2.5) for toxicodynamics. Generally, rodents metabolize chemicals at a faster rate than humans, and the subdivision is consistent with the approximately 4-fold difference between rats (the most commonly used test species) and humans in basic physiological parameters that are major determinants of clearance and elimination of chemicals, such as cardiac output and renal and liver blood flows. The limited

data analysed by Renwick (1993) indicated greater potential variability within humans in kinetics than in dynamics, so a larger factor was suggested for kinetic variability. In a subsequent review for a World Health Organization (WHO) Task Group on Environmental Health Criteria for Guidance Values for Human Exposure Limits (IPCS, 1994), it was concluded that the 4.0 × 2.5 subdivision of the interspecies factor was appropriate because it was based on underlying physiological differences between species. However, it was considered that the database analysed was insufficient to justify an uneven subdivision of the 10-fold factor for human variability, and therefore this factor was divided evenly into two subfactors each of $10^{0.5}$ (3.16). This equal subdivision of the human variability factor was supported by a subsequent, more extensive analysis of appropriate kinetic parameters for 60 chemicals in humans and concentration–effect data for 49 chemical-related effects (Renwick & Lazarus, 1998).

The database used to derive the values for the subfactors (Figure 2) related to systemic effects produced after oral or intravenous dosage, but the use of CSAFs and the approach described below are applicable also to effects at the site of contact, where the toxicokinetic component would be direct delivery and not via the general circulation. The validity of the default values for the subfactors for such contact effects needs to be evaluated.



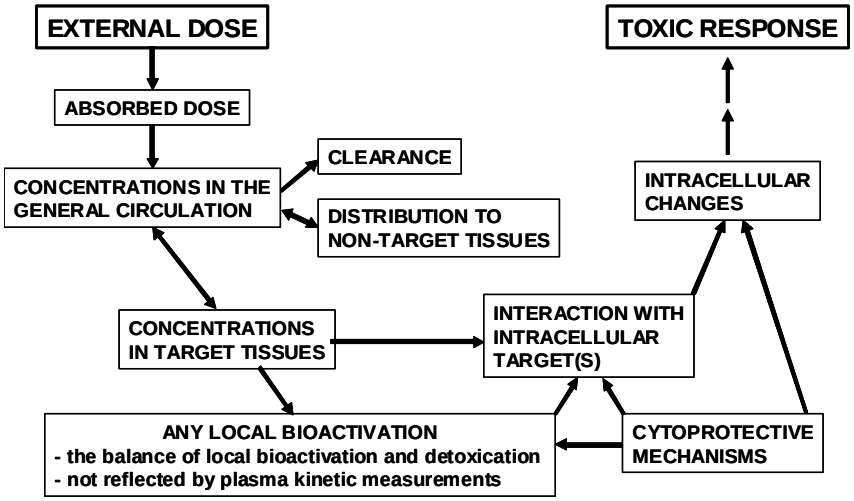
AD_{UF} = Uncertainty factor for animal to human differences in toxicodynamics
AK_{UF} = Uncertainty factor for animal to human differences in toxicokinetics
HD_{UF} = Uncertainty factor for human variability in toxicodynamics
HK_{UF} = Uncertainty factor for human variability in toxicokinetics

Figure 2. Subdivision of the usual uncertainty factor of 100 used in setting guidance values for the exposure of the general population, such as ADIs, TDIs or RfDs. Different numerical values could be derived if the usual total default uncertainty factor were not 100 — for example, in the risk assessment of occupational exposures (based on IPCS, 1994).

2.3 Separation of the overall process producing toxicity into toxicokinetics and toxicodynamics within the context of chemical-specific adjustment factors

Where there are adequate *in vivo* data in humans to characterize exposure–response in the population group of interest — for example, the general population or the critical susceptible subgroup thereof — these data would serve as the basis for development of tolerable, acceptable or reference concentrations or doses and would encompass both kinetic and dynamic aspects. Such *in vivo* response data for an adverse effect may be obtained from epidemiological studies — for example, in exposed workers. Ethical considerations mean that toxicity *per se* cannot be investigated directly in experimental studies with human volunteers. Mild and reversible biomarkers of the adverse effect may be studied, but this would require a clear understanding of the mode of action and the lack of health consequences at the doses studied (Renwick & Walton, 2001). Data addressing either kinetic or dynamic aspects in animals and humans for specific chemicals can be informative in quantitatively defining interspecies differences and interindividual variations in the human population.

As illustrated in Figure 3, there are a large number of steps on the pathway between administration of the external dose and the final toxic effect.



Note: "CONCENTRATIONS" refers to the relevant active form delivered by the general circulation and may be the parent compound or an active metabolite produced in another tissue and delivered to the target tissue or organ.

Figure 3. Processes leading to the generation of a toxic response.

For practical purposes, the continuous process between external dose and toxic response can be subdivided into steps related to the fate of the chemical in the body and those related to the actions of the chemical on the body. These different aspects of the overall process are termed toxicokinetics and toxicodynamics, respectively. They represent major sources of interspecies differences and of human variability, which can result in the existence of susceptible sub-groups within the population.

The metabolic and physiological processes involved in the absorption, distribution and elimination of foreign chemicals are similar, whether the chemicals are medicines, pesticides, food additives or industrial chemicals. In the context of this guidance document, "pharmacokinetic" and "toxicokinetic" can be considered to have the same meaning, and a "physiologically based pharmacokinetic (PBPK) model" is equivalent to a "physiologically based toxicokinetic (PBTK) model."

The data used to subdivide each factor of 10 into toxicokinetic and toxicodynamic aspects were derived largely from physiological differences between rodents and humans for the interspecies differences and from the clinical pharmacology literature for human variability, in which the variability in the kinetics data was based on plasma concentration measurements such as CL and AUC, whereas variability in the dynamics data was based on concentration–effect relationships derived from *in vitro* studies or from *in vivo* kinetic–dynamic modelling. In consequence, the data used to replace a default subfactor for toxicokinetics or toxicodynamics should be based on the concentrations of the chemical or active metabolite in the general circulation; if not, then the defaults for the remaining subfactors that were not replaced by a CSAF would need to be reconsidered.

2.3.1 Toxicokinetic data

Data on the absorption, distribution, metabolism and excretion of chemicals (ADME) are increasingly available as a basis for definition of plasma and tissue toxicokinetics. These data permit quantification of interspecies differences (i.e., differences between test species and humans) and variability among humans (i.e., differences between individuals and subgroups within the population) in the internal or target organ dose. Quantitative characterization in both of these areas requires the availability of toxicokinetic data for humans.

Ideally, both interspecies differences and human variability in toxicokinetics would be based on the free concentration of the active moiety in the target tissue/organ; however, because such data will be available only very rarely for humans, the guidance is based upon the use of readily available measurements that reflect the target tissue/organ concentration, such as the concentration of the active moiety in the general circulation. The concentrations and actions at the site of administration would be of relevance where the toxic response is at the site of contact.

Relevant data could be derived from *in vivo* studies that defined the kinetics of the chemical under the experimental conditions in animals and in humans at the anticipated human exposure dose or concentration. Because physiological and metabolic processes are independent of dose at low concentrations, appropriate toxicokinetic data may be derived ethically from *in vivo* experimentation in human volunteers given very low, non-toxic doses of the chemical under evaluation.

Any non-linearity in kinetics would need to be considered in the selection of the appropriate doses for assessment of either interspecies differences or human variability. The dose in animals should be that used as the basis for risk assessment — for example, the NOAEL for the critical effect in the pivotal study; if a different dose is used in the toxicokinetic study

used to derive a CSAF, then there should be consideration of whether non-linear kinetics could influence the parameter estimate. The dose that defines the toxicokinetics in humans should be that to which humans are exposed for an existing exposure or to which humans are predicted to be exposed when the chemical is subject to a prior approval procedure (Edler et al., 2002). In the latter case, the dose studied initially in humans could be 100-fold lower than the NOAEL in the animal study, but the possible impact of non-linear kinetics would need to be considered if a CSAF were derived from such data that gave a significantly different potential intake after application of the resulting CUF (see section 3.1.2) to the animal NOAEL.

In vitro measurements of critical processes (e.g., enzyme activity) can be used to estimate interspecies differences, especially when incorporated into a PBPK model (see below). In some cases, kinetic data can be obtained from measurements related to environmental exposures; however, exposure is often not adequately characterized for the individual or population as a whole. Such data may be informative, however, if the fate of the chemical is clearly understood based on studies in animals. For example, even without precise quantification of exposure, simple urine and plasma measurements are a reflection of total clearance for a substance that is eliminated largely via the kidneys.

Physiologically based kinetic parameters

The development of a PBPK model (see below) may not be necessary when physiologically based parameters such as CL and AUC can be derived from *in vivo* studies at appropriate doses. Estimates of CL can also be derived from *in vitro* enzyme studies combined with suitable scaling to determine *in vivo* activity or by the scaling of *in vivo* data from animals to predict human equivalent values (Obach et al., 1997). Plasma data will reflect the extent of partitioning of the chemical between the general circulation and the body tissues, but will not give a direct measure of the concentration in the target tissue/organ. In general, partitioning between plasma and tissues is by simple passive diffusion and is not usually a major source of either interspecies differences or interindividual variability; however, interspecies differences in tissue:plasma ratio could occur for chemicals that are highly bound to proteins or are substrates for transmembrane transporters. Physiologically based parameters are of value in relation to average steady-state concentrations in blood or plasma, but do not define tissue concentrations or the changes in tissue levels following a single dose.

Physiologically based pharmacokinetic models

A major advantage of PBPK models is that they can model the changes in the concentrations of the chemical or its active metabolite in body tissues, including the target tissue/organ, following a single exposure and also at steady state following repeated dosage. PBPK models can be helpful in extrapolations across routes and over dose ranges, particularly when metabolism or tissue uptake is non-linear. Partitioning of the chemical between the general circulation and target tissue/organ is usually based on measurements of the partition coefficient using animal tissues or other *in vitro* models. The data on partition coefficients for different tissues are combined with the organ blood flows for animals and for humans (see

Davies & Morris, 1993; Walton et al., 2004) to produce a PBPK model that can define delivery to and the concentrations within the target tissue/organ.

In the context of the present guidance, PBPK models for situations where the parent compound or a metabolite is delivered to the target tissue/organ via the general circulation may be subdivided into two types:

1) those that estimate the target tissue/organ dose of the parent compound or a circulating active metabolite; and
2) those that additionally incorporate bioactivation and detoxication processes that occur within the target tissue/organ.

Type 1 PBPK models are purely "toxicokinetic" in nature and would be appropriate for the types of data that were used to divide the uncertainty factors of 10 into toxicokinetic and toxicodynamic subfactors. Type 2 PBPK models include parts of the overall process (see Figure 3 above) that are not reflected in plasma-based toxicokinetic measurements and therefore reflect processes affecting the tissue "response" and are part of toxicodynamics. PBPK models that incorporate bioactivation within the target tissue/organ have been used most frequently in the context of extrapolation between species for direct-acting or genotoxic carcinogens.

In addition to these PBPK models for systemic delivery, mathematical models can define delivery when the target tissue/organ is the site of contact, such as inhalation delivery to the lungs, as well as uptake and metabolic processing within the target tissue/organ. Such models differ from the database used to derive the values for the subfactors given in Figure 2 (see section 2.2), and consideration would need to be given to the appropriateness of these values for the particular model on a case-by-case basis.

2.3.2 Toxicodynamic data

Toxicodynamic data are those that address any of the whole range of steps from molecular interaction (e.g., receptor binding) up to the effect at the target site. Whereas mode of action is understood for a range of effects induced by particular chemicals, a detailed knowledge of the mechanism of action is often not available (see section 1.3). There is usually also no detailed knowledge on how the dose/concentration–effect relationship of one step at the effect site is related to the dose/concentration–effect relationship of the next step. However, CSAFs for interspecies differences and human variability may be derived from comparative response data for the toxic effect itself in the target tissue/organ (e.g., haemolysis, as in Case B in Appendix 1) or for a point in the chain of events that is considered critical to the toxic response — for example, a key event, such as a precursor effect, which is based on understanding of mode of action (Sonich-Mullin et al., 2001). Such measurements should be derived under experimental conditions where variations in toxicokinetics have been precluded.

Hence, CSAFs could be derived from *in vitro* studies, from *in vivo* studies in which the toxicokinetic component has been delineated or from *ex vivo* experimentation (i.e., studies in

which measurements are made *in vitro* following an *in vivo* exposure). Some of the measurements of effect relate to biological responses that develop without a time lag after exposure. They could be reversible or irreversible. Some measures of response relate to biochemical changes (e.g., elevation of liver enzymes) that result from early histopathological changes after continuous administration of the chemical.

In kinetic–dynamic link models that must be externally validated, the estimated concentrations or amounts at the site of action are related to the response by an empirical mathematical link-function formula. Such models provide estimates of the concentration–response relationship excluding toxicokinetic aspects.

Not all *in vitro* or *in vivo* biological measurements represent processes that are critical to the development of the *in vivo* toxic response. There are frequently numerous sequential steps in producing a toxic response, and biomarkers of early changes may not reflect the critical toxicodynamic process. In order to serve as a surrogate marker for toxic effect, the measurements should be representative, both qualitatively and quantitatively, of the critical toxic endpoint. Incorporation of appropriate *in vitro* toxicodynamic data into considerations of interspecies differences in toxicodynamics could contribute to a reduction in *in vivo* animal studies. For the generation of a CSAF for interspecies differences, comparable *in vitro* studies would be required in both animal and human tissues (see section 3.2.3).

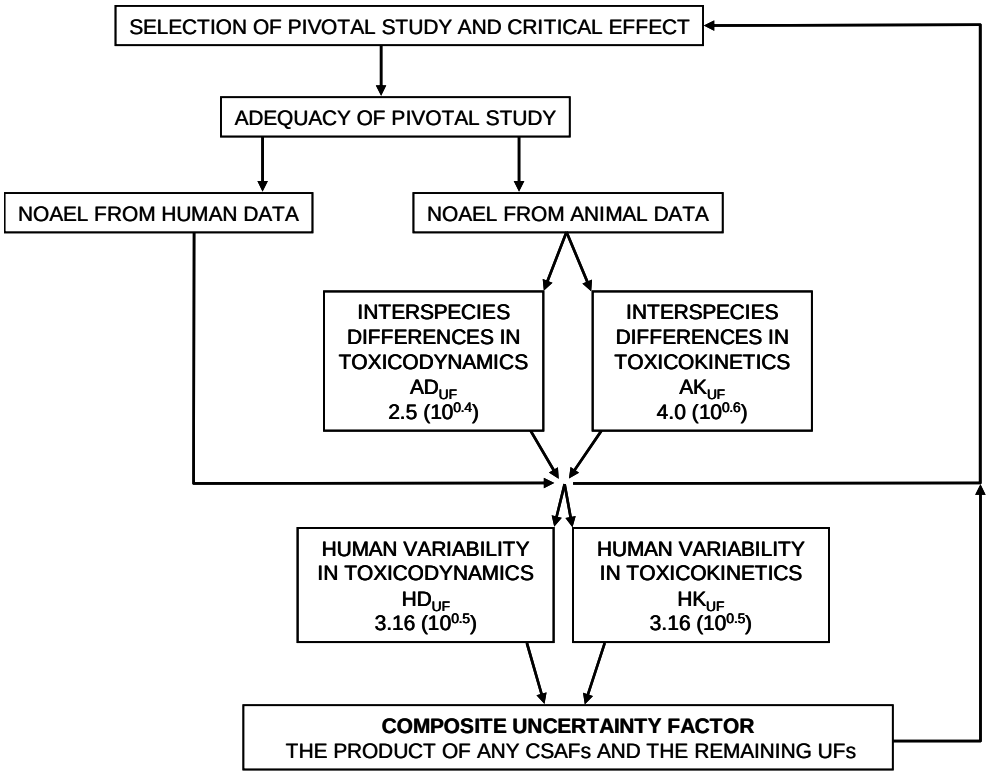
2.4 Calculation of the composite uncertainty factor

The scheme for dose/concentration–response assessment developed in IPCS (1994) is summarized below (Figure 4). The scheme is based on subdivision of the factors of 10 used for risk assessment for the general population and collapses back to the usual factor of 100 in the absence of appropriate data, but allows the potential for quantitative chemical-specific data to be introduced.

This procedure allows the replacement of part of the usual uncertainty factor of 100 used for risk assessment for the general population with quantitative chemical-specific data relating to either toxicokinetics or toxicodynamics. Such data will thereby have a direct and quantitative impact on the risk assessment outcome. The replacement of a default subfactor for either toxicokinetics or toxicodynamics with quantitative chemical-specific data will result in a CSAF for that particular aspect for which data were available. The CSAF is determined by the chemical-specific data and may be above or below the default; an interspecies factor could be less than 1 if humans had lower target tissue exposure to the active chemical moiety for the same external dose or showed lower tissue sensitivity. The use of chemical-specific data has the effect of replacing a default factor for an area of uncertainty with chemical-specific data, thereby reducing the overall uncertainty.

The CUF applied to the NOAEL or NOAEC (or BMD, BMC, LOAEL, LOAEC) is the composite of the CSAFs and any remaining default uncertainty factors for which appropriate chemical-specific data were not available:

$$CUF = [AK_{AF} \text{ or } AK_{UF}] \times [AD_{AF} \text{ or } AD_{UF}] \times [HK_{AF} \text{ or } HK_{UF}] \times [HD_{AF} \text{ or } HD_{UF}]$$



A = Animal to human comparison; H = Human variability; D = Toxicodynamics; K = Toxicokinetics; UF = Uncertainty factor (which would be replaced by a CSAF if suitable data were available)

Figure 4. Scheme for the introduction of quantitative toxicokinetic and toxicodynamic data into dose/concentration–response assessment (adapted from IPCS, 1994).

where:

- A represents the animal to human extrapolation factor (based on quantification of interspecies differences)
- H represents the human variability factor (based on quantification of interindividual differences)
- K stands for differences in toxicokinetics
- D stands for differences in toxicodynamics
- AF is the adjustment factor calculated from chemical-specific data
- UF is the uncertainty factor, a default value that is used in the absence of chemical-specific data.

The total CUF could be either greater than or less than the usual default (100 in Figures 2 and 4), depending on the quantitative scientific data that have been introduced to replace the default uncertainty factors. It is recognized in IPCS (1994) that the result of such replacement might be a CUF that is less than the normal default value. Whenever CSAFs are less than the default they replace, the dose/concentration–response assessment may need to be based on an

effect that occurs at a higher dose or concentration but for which relevant kinetic or dynamic data are not available as a basis for replacing the usual default (see Case A2 in Appendix 1), because a different toxic effect with a higher NOAEL/NOAEC combined with a standard default uncertainty factor could become the critical effect. This possibility also needs to be considered in designing studies to develop data as a basis for CSAFs. In consequence, arrows go back to the outset of the process in Figure 4 to ensure that all potential adverse effects and appropriate adjustment/uncertainty factors are considered adequately. The critical effect can be defined as *the first adverse effect, or its known precursor, that occurs in the increasing dose/concentration scale after appropriate adjustment for interspecies differences and interindividual variability*.

In the vast majority of cases, the quantitative toxicokinetic or toxicodynamic data necessary to define a CSAF will not be available, and hazard characterization will be necessary using the usual NOAEL/uncertainty factor approach. The default uncertainty factors (AK_{UF}, AD_{UF}, HK_{UF} and HD_{UF}) are based on the usual default values (10 for each of interspecies differences and human variability), so this guidance remains compatible with the current default procedures.

Because of the nature of the data on which the subdivision of the uncertainty factors is based, within the context of CSAFs, bioactivation in the target tissue represents the first step in the tissue response to the delivery of the chemical from the general circulation and therefore is considered the initial step in toxicodynamics. Thus, in the context of the subfactors for toxicokinetics and toxicodynamics, PBPK models that include target tissue bioactivation will include an initial part of the factor for toxicodynamics, and this will need to be considered on a case-by-case basis. For example, in some cases, tissue uptake is a function of the first step of bioactivation at the target site. The replacement of the interspecies toxicokinetics factor by parameters from PBPK models that include target organ bioactivation will take into account an undefined part of the interspecies toxicodynamics factor; the extent of this can be defined only by a full biologically based dose/concentration–response model, and this would replace both aspects — i.e., the original factor of 10 — when it is available.

3. GUIDANCE FOR THE USE OF DATA IN DEVELOPMENT OF CHEMICAL-SPECIFIC ADJUSTMENT FACTORS FOR INTERSPECIES DIFFERENCES AND HUMAN VARIABILITY

The following text is intended to provide practical guidance on the types and quality of data that could be used to derive a CSAF. The guidance is separated into five main sections:

- data related to interspecies differences in toxicokinetics
- data related to interspecies differences in toxicodynamics
- data related to human variability in toxicokinetics
- data related to human variability in toxicodynamics
- combination of adjustment factors and default uncertainty factors to derive a CUF.

The text for each section is self-contained, such that the adequacy of chemical-specific data related to any one of the possible default subfactors can be assessed without reference to the other subfactors that were not under consideration. Because of this, there is considerable intentional repetition in this section.

In the absence of adequate data to characterize the exposure–response relationship in humans that can be used to directly derive a health-based guidance value, such as an ADI, TDI or RfD, chemical-specific data on kinetic and dynamic aspects can be informative in quantitatively addressing interspecies and interindividual variation in the development of such acceptable, tolerable or reference concentrations or doses. In the following sections, guidance for the application of such data is provided in the context of replacing default values with those based on robust chemical-specific scientific data on kinetics and dynamics. Kinetic data are those considered to be related to delivery of the chemical to the target organ, and dynamic data are those related to concentration–response in the target tissue.

It should be recognized that while this guidance is presented in the context of the current default framework to address interspecies and interindividual variation using two factors of 10, this does not imply that these values are necessarily "correct," but rather permits incorporation of informative data on kinetics and dynamics, in the absence of a full biologically based dose–response model. It is anticipated that with increasing information on mode of action, reliance on factors of 10 to address these aspects will decrease. The framework also lends itself to presentation of dose–response in a probabilistic context, where data are sufficient to characterize the distributions of interest with confidence.

One of the first steps in implementation of this guidance is careful consideration of the relevant toxicokinetic parameter or the measure of effect for quantification of interspecies differences or human variability in toxicodynamics, sometimes referred to as a "metric," as a basis for CSAFs, in relation to the delivery of the chemical to the target tissue/organ. Measures of various end-points *in vivo* (i.e., biomarkers) may represent purely toxicokinetics or toxicokinetics and part or all of the toxicodynamic processes. CSAFs to replace the toxicokinetic default for interspecies differences or human variability can be based on measured parameters that reflect the internal dose (e.g., the concentration of a chemical in the circulation). *In vivo* measurements that incorporate some aspect of intracellular processing

related to the mode of action, such as formation of protein adducts or enzyme activation or inhibition, will reflect the uptake and delivery (kinetics) and at least part of the dynamics. Such a measurement could be used to replace the toxicokinetic default and a proportion of the toxicodynamic default for interspecies differences (see section 3.1 on AK_{AF}).

As discussed above, the division of the uncertainty factors into subfactors for toxicokinetics and toxicodynamics was based on concentrations of the parent or active metabolite in the general circulation, because this is what had been measured in the human studies that comprised the database. Metabolism within the target tissue/organ that cannot be estimated from blood measurements is therefore an early step in the overall toxicodynamic process. In some cases, the split between kinetics and dynamics in the framework may be difficult to define — for example, where metabolism in the target site may be important in both uptake into the target tissue (nominally kinetics) and the mode of action (nominally dynamics). In consequence, it may be necessary to determine the extent to which the available data, such as a PBPK model, cover part of the toxicodynamic process.

The basis for the subdivision of the original default factors of 10 was human pharmacokinetic–pharmacodynamic data primarily related to systemic exposure after oral and intravenous dosage and associated systemic effects. However, the contextual framework is equally applicable for other routes of exposure, such as inhalation, although defaults for components may vary somewhat. Thus, the approach is, for example, analogous to the reference concentration (RfC) methodology developed by the US Environmental Protection Agency for effects produced following inhalation exposure (US EPA, 1994).

The type of data that can be used to calculate a CSAF would not necessarily be part of a normal regulatory database, and there should be critical scientific evaluation of any information used. In reality, suitable data are likely to be available only for extensively studied chemicals. In cases where the main processes involved in either the toxicokinetics or toxicodynamics of the chemical are identified, but chemical-specific data from studies in humans are not available, it may be possible to use knowledge about interspecies differences or human variability in those processes to define an appropriate CSAF; the use of such pathway/process-related knowledge could be better than simply using the default value. An example of pathway/process-related knowledge would be interspecies differences and human variability in glomerular filtration rate, if this were the major determinant of internal dose.

An important aspect of the use of experimental data in the development of CSAFs is the recognition that the values used to calculate a CSAF will contain experimental or other errors and that these will affect the CSAF itself. In well designed and conducted studies, random experimental errors, but not systematic or study design errors, will not greatly influence the central tendency that is used for CSAFs related to interspecies differences. Errors in the mean parameter estimates used to derive an interspecies adjustment factor may make the calculated ratio either over- or underconservative, i.e., it could be over- or underprotective of human health. In contrast, any experimental errors that contribute to the apparent human variability used to derive a CSAF for human variability will result in a larger coefficient of variation and a larger than necessary factor and therefore will be conservative — i.e., tend to be overprotective. The use of CSAFs does not require data from multiple studies in humans if

suitable data of sufficient quality are available from a single study. If studies are to be performed specifically to produce data for the development of a CSAF, then the study design should take into account the different criteria given in the following sections (sections 3.1 to 3.4). The acceptability of the available data to derive a CSAF to replace a default uncertainty factor will be a matter of expert judgement and will require careful scrutiny on a case-by-case basis. It is not possible to give precise guidance that would be generally applicable, and the text given below indicates the considerations that would need to be taken into account by risk assessors when determining if a CSAF should be derived from the available data. The quality of the data will be critically important if the derived CSAF results in a total factor (CUF) of less than the usual default (100 in Figure 2).

The overall decision tree for the framework presented in Figure 4 is given in Figure 5. In the sections that follow (sections 3.1–3.4), guidance for the development of adjustment factors for interspecies differences and human variability is presented for each component of the framework. This guidance is also presented diagrammatically in Figures 6, 7, 8 and 10.

3.1 Data for the development of a chemical-specific adjustment factor for interspecies differences in toxicokinetics (AK_{AF}) (Figure 6)

3.1.1 Identification of the active chemical moiety

When applying the scheme to a specific chemical, the first step is to identify the active moiety — i.e., the parent compound or a metabolite that is responsible for the critical effect in question. If the data are not sufficient to draw a conclusion on the toxicologically active moiety, then the conventional default approach should be applied. If interspecies differences in the toxicokinetics of the parent compound or active metabolite(s) would lead to a CSAF that would exceed the default, then consideration should be given to using the higher value, even in the absence of data clearly defining the active chemical moiety, because the default might not be adequately protective.

There are several lines of evidence that may inform the determination of the active chemical moiety:

- The totality of the database on the chemical should be assessed for indications of the role of the parent compound or metabolite(s) in producing the critical toxic effect.
- Data on the mechanism of toxicity of structural analogues may indicate the likely active chemical moiety.
- If there is no metabolism, the critical effect is obviously caused by the parent compound.
- If the chemical is metabolized, then observation of the critical effect after administration of the metabolite(s) may allow identification of the active chemical moiety *in vivo* as well as *in vitro* (see Cases A1 and B in Appendix 1).
- In some cases, there may be data available on the influence of induction or inhibition of metabolism of the chemical on the critical effect. If inhibition (which lowers the clearance of the parent compound and increases its AUC/concentration) is followed by a decrease in the critical effect or decreased effect size, it is likely that the effect is caused by a

metabolite. If the effect increases, this is an indication that the active chemical moiety is likely to be the parent compound. The same changes after enzyme induction would lead to the opposite conclusions. The strength of such evidence would be increased by kinetic data demonstrating that the anticipated metabolic pattern occurs *in vivo*.

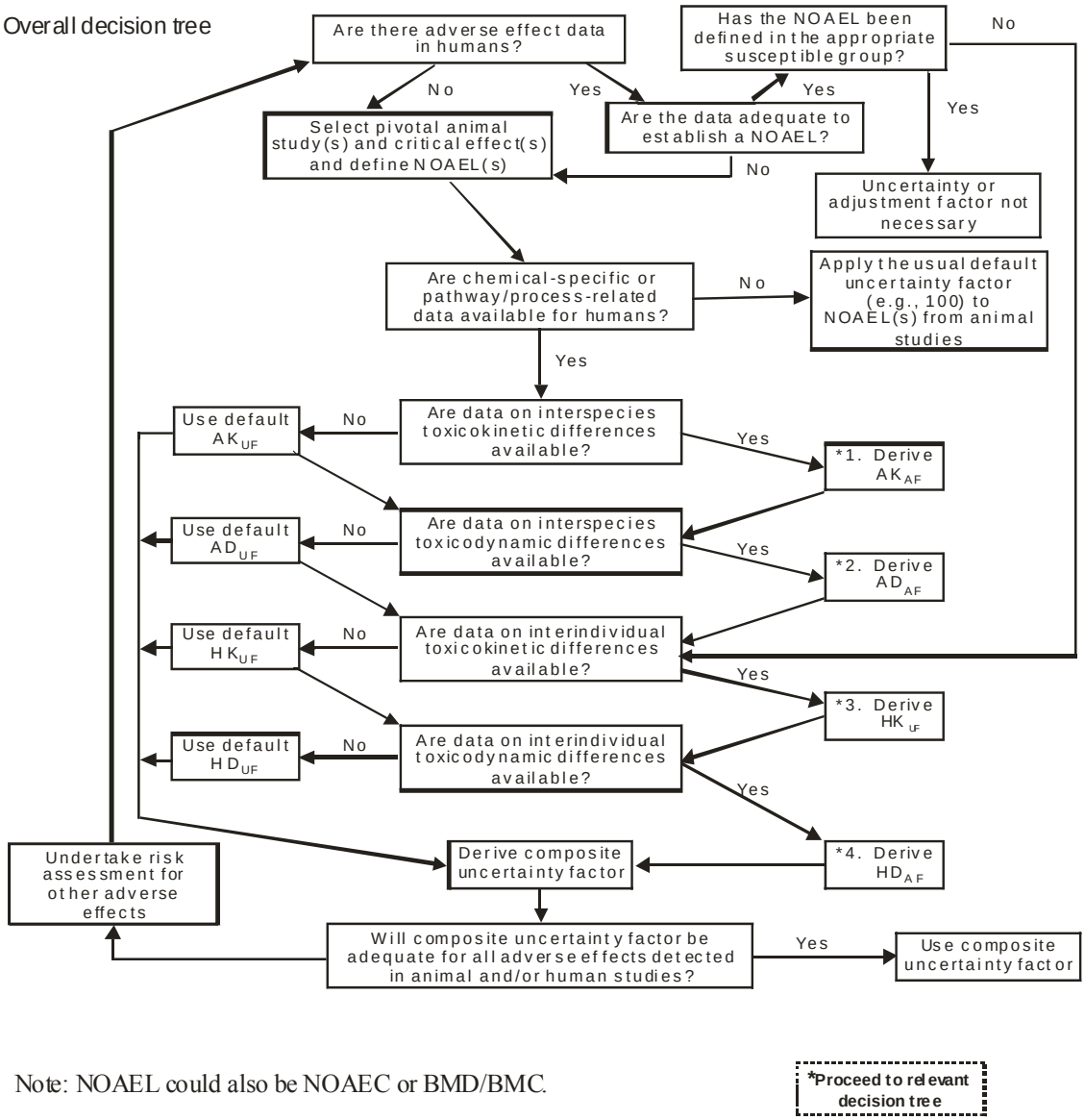


Figure 5. Overview for development of the CUF.

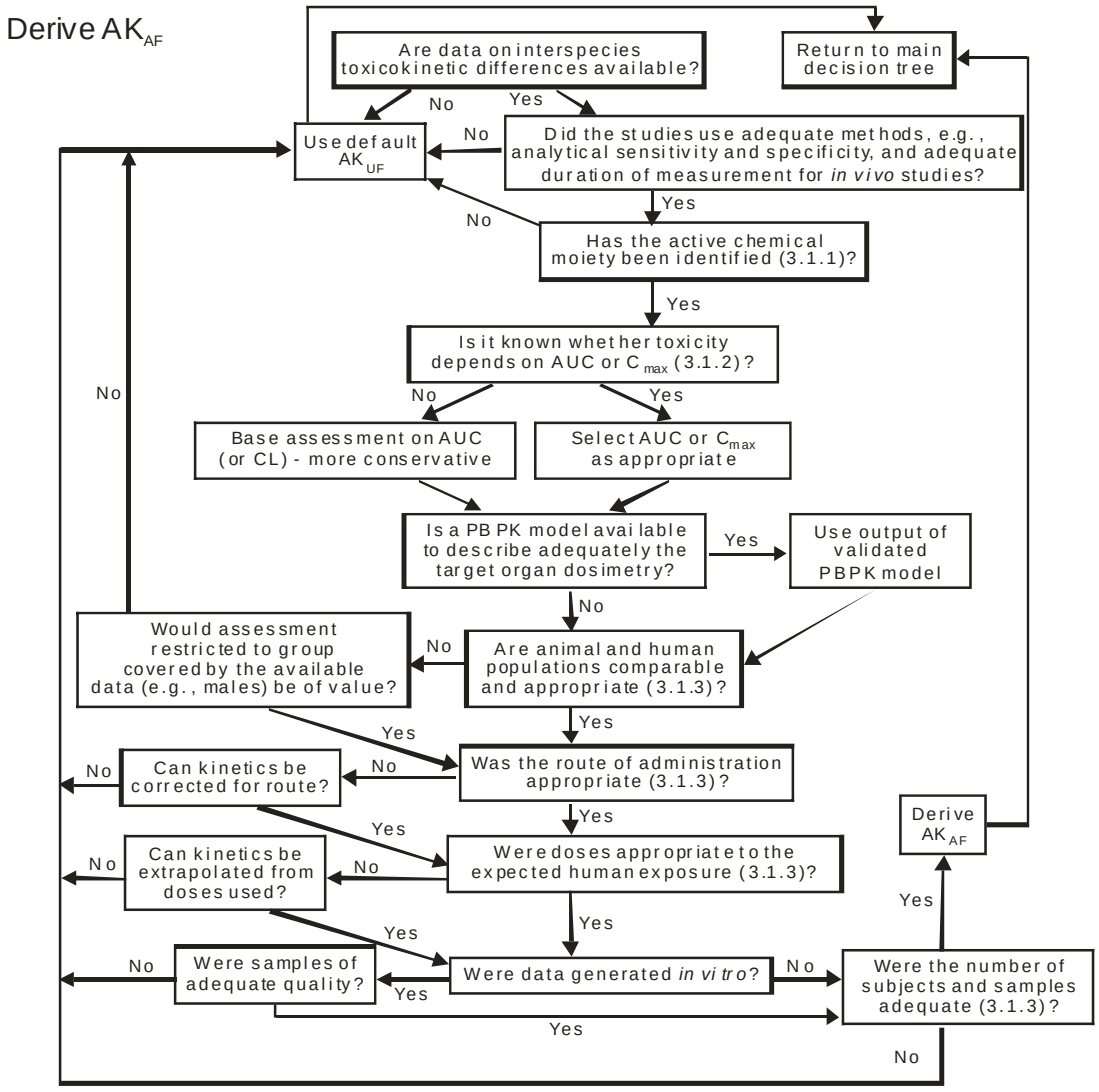


Figure 6. Derivation of AK_{AF} (see accompanying explanatory text).

- The importance of metabolism may be inferred by evaluation of the database on the chemical in relation to differences in dynamic response/critical effect. For example, there may be differences in response in relation to the species and strain studied, the route of administration (e.g., inhalation vs. oral) or the mode of administration (e.g., gavage vs. diet). Such observations should be supported by kinetic data on the parent compound and/or data on metabolism to determine whether the differences in response could be caused by different metabolic patterns; for example, first-pass metabolism could occur

after oral but not inhalation administration, while saturation of metabolism is more likely after bolus administration, such as gavage.

3.1.2 Choice of relevant toxicokinetic parameter

For toxicokinetics, an initial decision is whether the critical effect is likely to be related to the maximum concentration delivered to the target tissue/organ (C_{max}), to the overall exposure (as given by AUC or 1/CL), reflecting the amount delivered to the target tissue/organ during the dosage period, or to some other variable, such as the rate of change of concentration after a bolus dose for an acute effect. Several lines of evidence may inform this decision:

- The ratio reflecting the internal dose or target tissue/organ exposure should be expressed in the form of human/animal, correcting the kinetic parameter as appropriate or when necessary to reflect internal exposure (e.g., 1/CL), because AUC and CL are inversely related (CL = internal dose / AUC).
- Toxicological studies only rarely provide data that can be used to make a distinction between C_{max} and AUC as the relevant toxicokinetic parameter related to the critical effect. In some cases, however, the effect may only be present or may be greater after an intravenous bolus dose or administration by gavage compared with the response after the chemical is infused or administered in the diet or drinking-water. Such data may indicate that the dose rate is an important determinant of the magnitude of response and that C_{max} is the appropriate toxicokinetic parameter for the effect.
- A reasonable assumption is that effects resulting from subchronic or chronic exposure would normally be related to the AUC, especially for chemicals with long half-lives, whereas acute toxicity could be related to either the AUC or the C_{max}. C_{max} could be more relevant than AUC when a simple bimolecular interaction produces the effect. Examples include acute pharmacological effects as a consequence of receptor binding and inhibition of enzymes, such as the inhibition of cholinesterase by carbamates (JMPR, 2002, 2005), and the reaction can be described by a direct-effect model.
- In cases where the data are not sufficient to make a clear decision, then the AUC of the parent compound, 1/CL derived from either *in vivo* or *in vitro* data or 1/CL derived from a PBPK model should be used; such an approach would be protective, because there are likely to be greater interspecies differences in AUC or 1/CL than in C_{max}.
- The most suitable toxicokinetic parameter may be the AUC for a dose interval at steady state, which would be reached after five half-lives. In typical cases in which the chemical does not induce or inhibit its own metabolism, the AUC after administration of a single dose extrapolated to infinity is a suitable alternative to the AUC for a dose interval at steady state. The dose used for *in vivo* studies in animals should be that used as the basis for risk assessment — for example, the NOAEL for the critical effect in the pivotal study; if a different dose is used in the toxicokinetic study used to derive a CSAF, then there should be consideration of whether non-linear kinetics could influence the parameter estimate. The dose that defines the toxicokinetics in humans should be that to which humans are exposed for an existing exposure or to which humans are predicted to be exposed when the chemical is subject to a prior approval procedure (Edler et al., 2002). In the latter case, the dose studied initially in humans could be 100-fold lower than the NOAEL in the animal study, but the possible impact of non-linear kinetics would need to

be considered if a CSAF were derived that resulted in a significantly different potential intake after application of the resulting CUF to the animal NOAEL.

- The AUC is the integral of concentration over time and can be derived from *in vivo* data. If AUC in plasma or target tissue/organ is used, then it should be corrected for dose and the CSAF calculated such that the value represents the human internal exposure divided by the animal internal exposure for the same external dose: for example, [(ng/ml)·h of chemical in human plasma per mg/kg body weight dosed divided by (ng/ml)·h in animal plasma per mg/kg body weight dosed]. If CL is the appropriate parameter, then the CSAF should be CL in animals divided by CL in humans, which will reflect the extent to which the animals clear the chemical more quickly than humans.
- *In vitro* data on enzyme activity from animal and human tissues can represent an important source of relevant information. However, enzyme kinetics should not be used directly, but should be scaled to determine the intrinsic clearance from the maximum metabolic rate (V_{max}) and the Michaelis-Menten kinetic constant (K_m) or incorporated into a PBPK model. (Case C in Appendix 1 illustrates this point, because the *in vivo* clearance is determined largely by liver blood flow and not by the enzyme activity measured *in vitro*.)
- *In vitro* data scaled to determine the intrinsic clearance from V_{max} and K_m can be used directly to predict whether the *in vivo* clearance may be determined by enzyme activity or by organ blood flow.
- If a PBPK model is used, the selected parameter should reflect, for example, C_{max} or AUC either in the plasma or better still in the target tissue/organ.
- PBPK models allow estimation of the target tissue/organ concentration–time curve and reflect non-linearity in tissue uptake if data defining the characteristics of a transmembrane transporter are incorporated into the model.
- If the plasma or target tissue/organ concentration is derived for the test species using a PBPK model or *in vitro* data scaled to *in vivo* clearance, then the same model with appropriate data could be used for deriving the same parameters for humans. An advantage of such an approach is that any errors in the model would apply to both species, but the output should be validated against any existing data to define the magnitude of any errors in the model.
- PBPK models should be validated; data sets on which the parameters are based are inappropriate as a basis for validation of the model.
- PBPK models, which include estimates of species differences in bioactivation within the target tissue/organ, address steps of the process leading to the tissue response, which is part of the toxicodynamics in the present context. If PBPK-based estimates of target tissue/organ exposure to the active metabolite (produced in the target tissue/organ) are used to replace the toxicokinetic default (AK_{UF}) of 4.0 by a CSAF (AK_{AF}), then an aspect of toxicodynamics will also have been addressed. If target tissue/organ exposure to the active metabolite is the basis for the kinetic adjustment factor (AK_{AF}) in combination with the full toxicodynamic default (AD_{UF}), then the composite interspecies factor ($AK_{AF} \times AD_{UF}$) will be conservative. Under such circumstances, it would be logical for the toxicodynamic default uncertainty factor to be reduced, but resolving the magnitude of any reduction will involve the development of a biologically based dose–response model (which could then be used to replace both interspecies factors). Without such data, the default AD_{UF} should be used.

3.1.3 Experimental data

Determination of the adequacy of the experimental data as a basis for the derivation of a CSAF is made on a case-by-case basis, taking into account a number of aspects of the critical studies, including nature of the population, relevance of the route of administration, doses administered and sample size.

1) *Relevance of population:*
   - The humans in the toxicokinetic study should be sufficiently representative of the population at risk of the adverse effect detected in the animal studies. For example, while the critical effect in Case A is testicular toxicity, kinetic data from females are appropriate for use, since sex-dependent variations are minor.
   - Ideally, the humans should be of an equivalent age or stage of development to the animals in which the adverse effects were observed; if not, the impact of any discrepancy on the validity of the calculated ratio for susceptible to average humans should be considered.
   - Data for potentially susceptible subgroups of the human population would not normally be used for quantitative adjustments for interspecies differences, because the difference between such groups and the general population should be incorporated under human variability (see section 3.3.3).

2) *Relevance of route:*
   - Ideally, *in vivo* kinetic studies in animals and humans should be performed via the route by which humans are normally exposed.
   - If the route for the kinetic studies in either animals or humans varies from that on which the critical effect level or BMD/BMC is based, then route-to-route extrapolation will be necessary, and the data will need to be assessed critically in relation to their use for the development of a CSAF. PBPK models are often informative in this context.

3) *Relevance of dose or concentration:*
   - Ideally, CSAFs for toxicokinetics should be based on data from animals exposed to doses/concentrations equivalent or similar to the BMD/BMC or the NOAEL/NOAEC and under dosage conditions similar to those in the toxicity study(ies) on which the BMD or the NOAEL is based. Any discrepancies should be assessed for their potential impact on the dose metric and the validity of the resulting AK_{AF}.
   - Ideally, the doses given in human studies should be similar to the estimated or potential human exposure. If not, the kinetic data should be assessed to determine if they are relevant to the levels of human exposure. The dose selected to calculate the AK_{AF} may need to be reconsidered if the CSAF is considerably different from the default.

4) *Adequacy of number of subjects/samples:*
   - The numbers of animals and humans should be sufficient to ensure that the data allow a reliable estimate of the central tendency for each species.

- The distribution of the data should be examined for evidence of any discontinuity. In practice, the presence of a small number of outliers would not be considered evidence of a discontinuous distribution. Where there is clear evidence of a discontinuous distribution, such as a genetic polymorphism, the adjustment factor should be based on the central tendency of the higher-frequency group, because subgroups of the population should be taken into account under human variability (see section 3.3.3).
- In the absence of evidence to the contrary, it can be assumed that the activities of the underlying elimination processes are normally or lognormally distributed within the sample population or within the major mode when the sample population is distributed discontinuously, as appropriate.
- The central tendency should be estimated as the simple arithmetic mean of the relevant data, transformed if necessary to allow for the known distribution of the relevant toxicokinetic parameter (e.g., AUC is lognormally distributed).
- The number of subjects within the population, or within the major subgroup if there are two or more groups, should be sufficient to provide an accurate measure of the central tendency. As a guide, the standard error (standard deviation [SD] of the sample divided by the square root of the sample size) should be less than approximately 20% of the mean. Based on available data, this would normally involve a minimum number of approximately five subjects or samples from five individuals, unless the variability is very low (i.e., small coefficient of variability).

5) *Additional consideration for* in vitro *studies:*
   - The quality of the samples should be considered and evidence provided that they are representative of the target population (e.g., viability of the tissue sample, specific content or activity of marker enzymes).

3.2 Data for the development of a chemical-specific adjustment factor for interspecies differences in toxicodynamics (AD_{AF}) (Figure 7)

Adjustment factors for interspecies toxicodynamic aspects will usually be based on results of *in vitro* studies using animal and human tissue, because if there are adequate *in vivo* data in humans, the measure of dose–response (i.e., effect level or BMD) would generally be used directly to define the NOAEL, such that there would be no need to extrapolate from *in vivo* animal data using an interspecies adjustment factor. Interspecies comparisons based on *in vivo* dose–response data would incorporate both toxicokinetic and toxicodynamic differences and would not be appropriate for replacing the interspecies toxicodynamic default factor (2.5).

3.2.1 Identification of the active chemical moiety

When applying the scheme to a specific chemical, the first step is to identify the active moiety — i.e., the parent compound or a metabolite that is responsible for the critical effect in question. If the data are not sufficient to draw a conclusion on the toxicologically active species, then the conventional default approach should be applied. If interspecies differences in the toxicodynamics of the parent compound or active metabolite would lead to a CSAF

that exceeded the default uncertainty factor, then consideration should be given to using the higher value, even in the absence of data clearly defining the active chemical moiety, because the default might not be adequately protective.

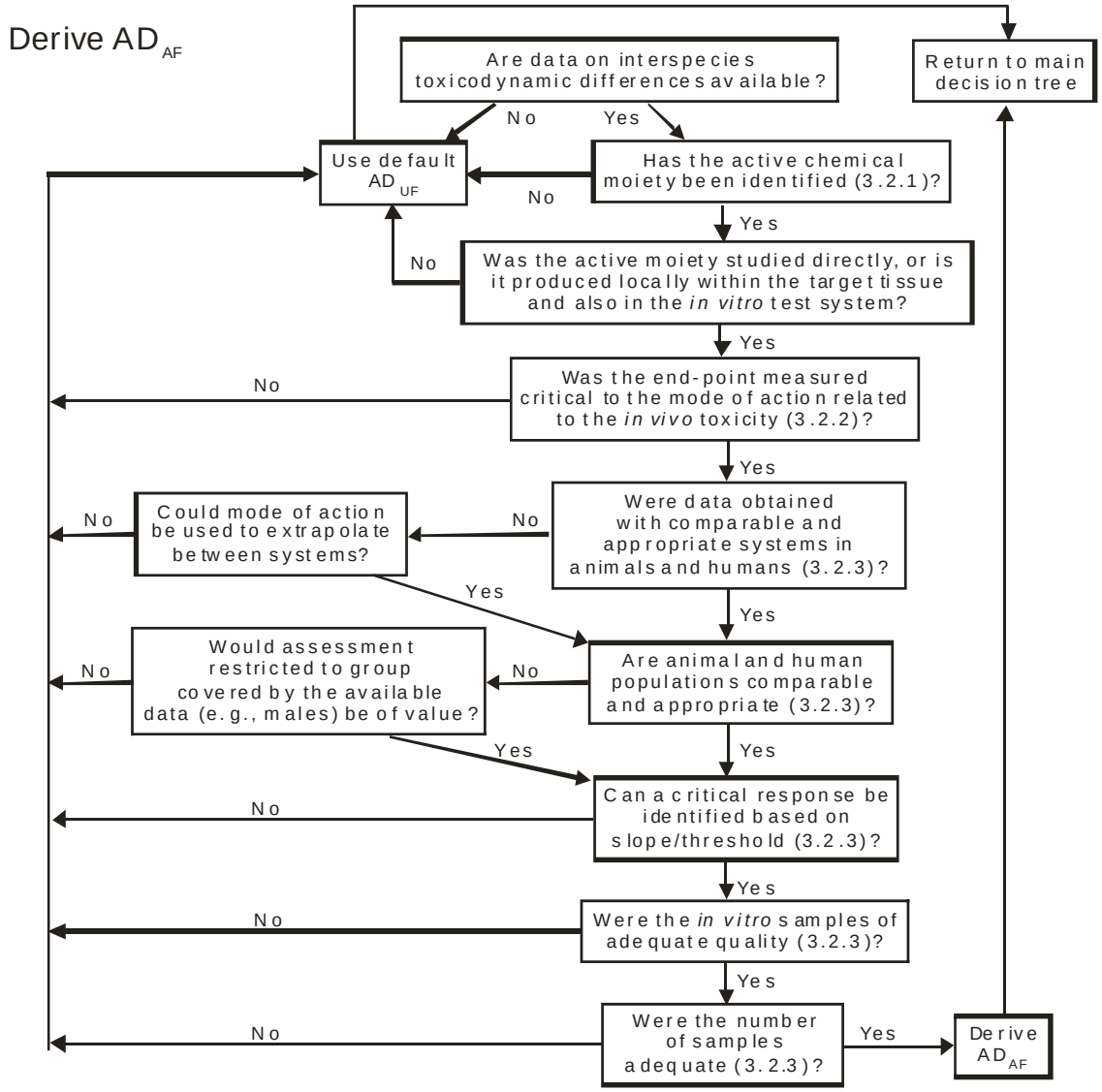


Figure 7. Derivation of AD_{AF} (see accompanying explanatory text).

There are several lines of evidence that may inform the identification of the active chemical moiety:

- The totality of the database on the chemical should be assessed for indications of the role of the parent compound or metabolite(s) in producing the critical toxic effect.
- Data on the mechanism of toxicity of structural analogues may indicate the likely active chemical moiety.
- If there is no metabolism, the critical effect is obviously caused by the parent compound.
- If the chemical is metabolized, then observation of the critical effect after administration of the metabolite(s) may allow identification of the active chemical moiety *in vivo* as well as *in vitro* (see Cases A1 and B in Appendix 1).
- In some cases, there may be data available on the influence of induction or inhibition of metabolism of the chemical on the critical effect. If inhibition (which lowers the clearance of the parent compound and increases its AUC/concentration) is followed by a decrease in the critical effect / decreased effect size, it is likely that the effect is caused by a metabolite. If the effect increases, this is an indication that the active chemical moiety is likely to be the parent compound. The same changes after enzyme induction would lead to the opposite conclusions. The strength of such evidence would be increased by kinetic data demonstrating that the anticipated metabolic pattern occurs *in vivo*.
- The importance of metabolism may be inferred by evaluation of the database on the chemical in relation to differences in dynamic response / critical effect. For example, there may be differences in response in relation to the species and strain studied, the route of administration (e.g., inhalation vs. oral) or the mode of administration (e.g., gavage vs. diet). Such observations should be supported by kinetic data on the parent compound and/or data on metabolism to determine whether the differences in response could be caused by different metabolic patterns; for example, first-pass metabolism could occur after oral but not inhalation administration, while saturation of metabolism is more likely after bolus administration, such as gavage.
- The active chemical moiety must be used in the relevant *in vitro* studies and/or there must be adequate metabolic capacity in the test system for the relevant bioactivation pathway.

3.2.2 Consideration of end-point

The end-point measured should be either the critical effect or a key event. Key events or surrogates are those that are intimately linked to the critical toxic effect based on understanding of mode of action. Dose–response and temporal relationships for key events/surrogates should be consistent with those for the critical toxic effect.

In vitro studies of the toxic response or a surrogate for the toxic end-point in animal and human tissues could provide relevant toxicodynamic data as a basis for development of the AD_{AF}. Such data will define target site sensitivity directly, without any toxicokinetic influences (see Case B in Appendix 1).

3.2.3 Experimental data

Determination of the adequacy of the experimental data as a basis for replacement of the default is made on a case-by-case basis, taking into account a number of aspects of the critical studies, including nature of the population, the concentration–response data and sample size.

1) *Relevance of population:*
   - The humans who were the source of tissue for *in vitro* study should be sufficiently representative of the population at risk of the adverse effect detected in the animal studies.
   - Ideally, the humans should be of an equivalent age or stage of development to the animals in which the critical adverse effects were observed; if not, the impact of any discrepancy on the validity of the calculated ratio should be considered.
   - Data for potentially susceptible subgroups of the human population should not be used for quantitative adjustments for interspecies differences, because the difference between such groups and the general population should be incorporated under human variability (see section 3.4.3).

2) *Adequacy of concentration–response data:*
   - Studies must be designed to include a suitable number of concentrations to adequately characterize the concentration–response in humans compared with the test species.
   - Quantitative comparisons of *in vitro* data for replacement of the dynamic component of the default value for interspecies differences should be based on concentrations that induce an effect of defined magnitude in both test species and humans (e.g., EC_{10}). They cannot be calculated from differences in the magnitude of the response in animal tissues and human tissues to the same concentration, because the difference ratio will most likely vary with concentration.
   - The experimental methods measuring the concentration–response relationship in animals and humans should be comparable in order to allow quantitative comparison.
   - Where the concentration–response curves in animals and humans are parallel, selection of the point for quantitative comparison (the metric) can be anywhere between 10% and 90% response on the concentration–response curve.
   - Where the curves are not parallel, the point for comparison should be the lowest point on the concentration–response curve that provides reliable information without extrapolation below the experimental data (e.g., EC_{10}).
   - At its simplest, the replacement of the default factor using *in vitro* data for inter-species differences in dynamics can be the ratio of these two measurements (e.g., the ratio of average animal to average human EC_{10} values). This is the correct form for the ratio, because a value greater than 1 would result if the EC_{10} for humans is lower, i.e., humans are more sensitive.

3) *Adequacy of number of subjects/samples:*
   - The numbers of animals and humans should be sufficient to ensure that the data allow a reliable estimate of the central tendency for each species.
   - The distribution of the data should be examined for evidence of any discontinuity. In practice, the presence of a small number of outliers would not be considered evidence of a discontinuous distribution. Where there is clear evidence of a discontinuous distribution — for example, due to a genetic polymorphism — the adjustment factor should be based on the central tendency of the higher-frequency group, because subgroups of the population should be taken into account under human variability (see section 3.4.3).

- In the absence of evidence to the contrary, it can be assumed that the activities of the underlying toxicodynamic processes are normally or lognormally distributed within the sample population or within the major mode when the sample population is distributed discontinuously, as appropriate.
- The central tendency should be estimated as the simple arithmetic mean of the relevant data, transformed if necessary to allow for the known distribution of the end-point.
- The number of subjects within the population, or within the major subgroup if there are two or more groups, should be sufficient to provide an accurate measure of the central tendency. As a guide, the standard error (standard deviation [SD] of the sample divided by the square root of the sample size) should be less than approximately 20% of the mean. Based on available data, this would normally involve a minimum number of approximately five subjects or samples from five individuals, unless the variability is very low (i.e., small coefficient of variability).

4) *Additional considerations for* in vitro *studies:*
   - The quality of the samples should be considered and evidence provided that they are representative of the target population (e.g., viability, specific content or activity of marker enzymes).
   - When limited *in vivo* data in humans are available, although they may be inadequate for direct use in characterization of concentration–response, they can be of value to check that the results of *in vitro* studies used for the development of the AD_{AF} are plausible.

3.3 Data for the development of a chemical-specific adjustment factor for human variability in toxicokinetics (HK_{AF}) (Figure 8)

3.3.1 Identification of the active chemical moiety

When applying the scheme to a specific chemical, the first step is to identify the active moiety — i.e., the parent compound or a metabolite responsible for the critical effect in question. If the data are not sufficient to draw a conclusion on the toxicologically active moiety, then the conventional default approach should be applied. The default uncertainty factor assumes that humans are more sensitive than the test species, but the reverse might be true if the effect were due to a metabolite.

There are several lines of evidence that may inform the determination of the active chemical moiety:

- The totality of the database on the chemical should be assessed for indications of the role of the parent compound or metabolite(s) in producing the critical toxic effect.
- Data on the mechanism of toxicity of structural analogues may indicate the likely active chemical moiety.
- If there is no metabolism, the critical effect is obviously caused by the parent compound.

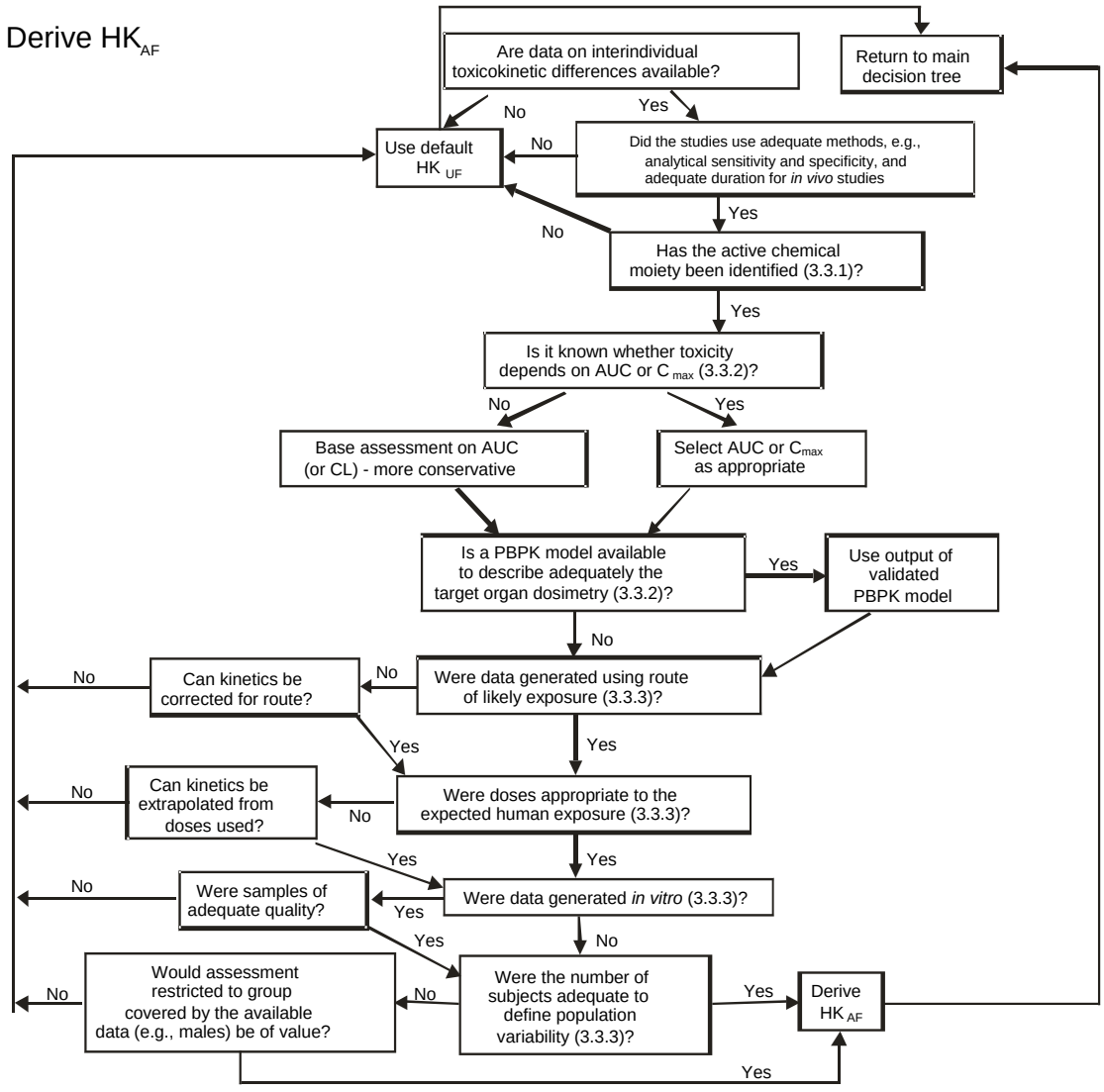


Figure 8. Derivation of HK_{AF} (see accompanying explanatory text).

- If the chemical is metabolized, then observation of the critical effect after administration of the metabolite(s) may allow identification of the active chemical moiety *in vivo* as well as *in vitro* (see Cases A1 and B in Appendix 1).
- In some cases, there may be data available on the influence of induction or inhibition of metabolism of the chemical on the critical effect. If inhibition (which lowers the clearance of the parent compound and increases its AUC/concentration) is followed by a decrease in the critical effect / decreased effect size, it is likely that the effect is caused by a metabolite. If the effect increases, this is an indication that the active chemical moiety is likely to be the parent compound. The same changes after enzyme induction would lead

to the opposite conclusions. The strength of such evidence would be increased by kinetic data demonstrating that the anticipated metabolic pattern occurs *in vivo*.

- The importance of metabolism may be inferred by evaluation of the database on the chemical in relation to differences in dynamic response / critical effect. For example, there may be differences in response in relation to the species and strain studied, the route of administration (e.g., inhalation vs. oral) or the mode of administration (e.g., gavage vs. diet). Such observations should be supported by kinetic data on the parent compound and/or data on metabolism to determine whether the differences in response could be caused by different metabolic patterns; for example, first-pass metabolism could occur after oral but not inhalation administration, while saturation of metabolism is more likely after bolus administration, such as gavage.

3.3.2 Choice of relevant toxicokinetic parameter

For toxicokinetics, an initial decision is whether the critical effect is likely to be related to the maximum concentration delivered to the target organ (C_{max}), to the overall exposure (as given by AUC or 1/CL), reflecting the amount delivered to the target organ during the dosage period, or to some other variable, such as the rate of change of concentration after a bolus dose for an acute effect. Several lines of evidence may inform this decision:

- The ratio reflecting the internal dose or target organ exposure should be expressed in the form of susceptible human / average human, correcting the kinetic parameter as appropriate or when necessary to reflect internal exposure (e.g., 1/CL), because AUC and CL are inversely related (CL = internal dose / AUC).
- Toxicological studies only rarely provide data that can be used to make a distinction between C_{max} and AUC as the relevant toxicokinetic parameter related to the critical effect. In some cases, however, the effect may only be present or may be greater after an intravenous bolus dose or administration by gavage compared with the response after the chemical is infused or administered in the diet or drinking-water. Such data may indicate that the dose rate is an important determinant for the magnitude of response and that C_{max} is the appropriate toxicokinetic parameter for the effect.
- A reasonable assumption is that effects resulting from subchronic or chronic exposure would normally be related to the AUC, especially for chemicals with long half-lives, whereas acute toxicity can be related to either the AUC or the C_{max}. C_{max} could be more relevant than AUC when a simple bimolecular interaction produces the effect. Examples include acute pharmacological effect as a consequence of receptor binding and inhibition of enzymes, such as the inhibition of cholinesterase by carbamates (JMPR, 2002, 2005), and the reaction can be described by a direct-effect model.
- In cases where the data are not sufficient to make a clear decision, then the AUC of the parent compound or 1/CL derived from either *in vivo* or *in vitro* data should be used; such an approach would be protective, because there is likely to be greater human variability in AUC or 1/CL than in C_{max}.
- The most suitable toxicokinetic parameter may be the AUC for a dose interval at steady state, which would be reached after five half-lives. In typical cases in which the chemical does not induce or inhibit its own metabolism, the AUC after administration of a single dose extrapolated to infinity is a suitable alternative to the AUC for a dose interval at

steady state. The dose that defines the toxicokinetics in humans should be that to which humans are exposed for an existing exposure or to which humans are predicted to be exposed when the chemical is subject to a prior approval procedure (Edler et al., 2002). In the latter case, the dose studied initially in humans could be 100-fold lower than the NOAEL in the animal study, but the possible impact of non-linear kinetics would need to be considered if a CSAF were derived that resulted in a significantly different potential intake after application of the resulting CUF to the animal NOAEL.

- The AUC is the integral of concentration over time and can be derived from *in vivo* data.
- *In vitro* data on enzyme activity from human tissues can represent an important source of relevant information. However, enzyme kinetics should not be used as such, but should be scaled to determine the intrinsic clearance from V_{max} and K_m or incorporated into a PBPK model. (Case C in Appendix 1 illustrates this point, because the *in vivo* clearance is determined largely by liver blood flow and not by the enzyme activity measured *in vitro*.)
- *In vitro* data scaled to determine the intrinsic clearance from V_{max} and K_m can be used directly to predict whether the *in vivo* clearance may be determined by enzyme activity or by organ blood flow.
- If a PBPK model is used, sources of human variability affecting the selected parameter — for example, C_{max} or AUC either in the plasma or in the target tissue/organ — should be incorporated into the model. It might be possible to use probabilistic approaches to define the range of human variability, including potentially susceptible subgroups — for example, by the incorporation of a population distribution for each parameter in the model — rather than a single average value, without the need for direct experimentation to define the population distribution.
- Where possible, PBPK models should be validated with data from humans that were not used to generate the model parameters.
- If the plasma or target tissue/organ concentration in the major population group is derived using a PBPK model or *in vitro* data scaled to *in vivo* clearance, parameters for any subgroups of the human population can be based on the same model.
- PBPK models that include estimates of human variability in bioactivation within the target tissue/organ address steps of the process leading to the tissue response, which is part of the toxicodynamics in the present context. If PBPK-based estimates of target tissue/organ exposure to the active metabolite (produced in the target tissue/organ) are used to replace the toxicokinetic default (HK_{UF}) by a CSAF (HK_{AF}), then an aspect of toxicodynamics will also have been addressed. If target tissue/organ exposure to the active metabolite is the basis for the kinetic adjustment factor (HK_{AF}) in combination with the full toxicodynamic default (HD_{UF}), then the composite interindividual factor ($HK_{AF} \times HD_{UF}$) will be more conservative. Under such circumstances, it would be logical for the toxicodynamic default uncertainty factor to be reduced, but resolving the magnitude of any reduction will involve the development of a biologically based dose–response model (which should then be used to replace both human variability factors). Without such data, the default HD_{UF} should be used.

3.3.3 Experimental data

Ideally, factors responsible for the clearance mechanisms should be identified (e.g., renal clearance, cytochrome P450 [CYP]-specific metabolism, etc.). In many cases, it may not be

practicable or possible to obtain toxicokinetic measurements on the specific chemical in a sufficient number of subjects to define human variability. However, if the major determinants of absorption and clearance are defined, then it may be possible to derive a CSAF based on known human variability in the relevant physiological and biochemical parameters (i.e., a chemical-related factor) (see Case A in Appendix 1).

Determination of the adequacy of the experimental data as a basis for replacement of the default is made on a case-by-case basis, taking into account a number of aspects of the critical studies, including nature of the population, relevance of the route of administration, doses administered and sample size.

1) *Relevance of population:*
   - The humans in the toxicokinetic study should be sufficiently representative of the complete population at risk of the adverse effect detected in the animal studies (e.g., relevant age groups and sexes). In cases where the available *in vivo* data relate to only one population group (e.g., adult males), the extent to which the toxicokinetic data may differ for other exposed groups (e.g., women and children) should be given careful consideration; in the absence of chemical-specific data, it might be possible to deduce variability in the other groups from knowledge of differences in the metabolic and physiological processes involved in the kinetics of the chemical. Human variability in many of the pathways of foreign compound elimination can be obtained from the studies of Dorne et al. (2002, 2005) or from kinetic simulation software.

2) *Relevance of route:*
   - Ideally, *in vivo* kinetic studies should be performed in humans via the same route of exposure as that in the toxicity study from which the critical effect level (NOAEL, NOAEC, BMD, BMC) was derived (which should also be the route by which humans are normally exposed).
   - If the kinetic data in humans are derived following exposure by a route other than that in the toxicity study on which the critical effect level or BMD/BMC is based, then route-to-route extrapolation will be necessary, and the data will need to be assessed critically in relation to their use for the development of a CSAF. PBPK models are often informative in this context.

3) *Relevance of dose:*
   - Ideally, the doses given in human studies should be similar to the estimated or potential human exposure. If not, the kinetic data should be assessed to determine if they are relevant to the levels of human exposure. The dose selected to calculate the HK_{AF} may need to be reconsidered if the CSAF is considerably different from the default.

4) *Adequacy of number of subjects/samples*:
   - The numbers of humans should be sufficient to ensure that the data allow a reliable estimate of the central tendency and of the population distribution for all potentially exposed groups (see Case A in Appendix 1).

- The distribution of the data should be examined for evidence of any discontinuity, indicating a distinct population subgroup. In practice, the presence of a small number of outliers would not be considered evidence of a discontinuous distribution. Where there is evidence of a discontinuous distribution, the population size should be sufficient to provide information on the distribution of the relevant toxicokinetic parameter in both the higher-frequency group and the subgroups.
- An understanding of the biochemical or physiological basis for the presence of a subgroup (polymorphism) can greatly assist in the design of studies to generate and interpret appropriate data.
- Data on the nature of the population distribution of measurements of the relevant toxicokinetic parameter should be used when available.
- In the absence of data on the nature of the population distribution and in the absence of other evidence to the contrary, it can be assumed that the activity of the underlying elimination process is lognormally distributed within the sample population (because it reflects many toxicokinetic parameters and is a more conservative assumption).
- The central tendency should be estimated as the simple geometric (or arithmetic, if transformed appropriately) mean of the relevant data.
- In general, characterization of variability will relate to the general population (i.e., all age groups). However, where there are potentially susceptible population subgroups, it is recommended that they be addressed separately, with a clear indication of the proportion of the population likely to be susceptible, as a basis for decision-making by risk managers.
- The population distribution should be analysed and the CSAF (HK_{AF}) calculated as the ratio between given percentiles (such as 95th, 97.5th and 99th) and the central tendency for the whole population (Figure 9, left-hand side). Alternatively, where there are susceptible subgroups, this ratio is the upper percentile for the susceptible subgroup and the central tendency for the remainder of the population (Figure 9, right-hand side). Where there are discrete subgroups of the population, the CSAFs for different percentiles should be calculated based on data for the whole population, including the subgroup, and also for the subgroup separately; both sets of results should be provided to the risk manager. If there is a polymorphism in the main pathway of elimination, the susceptible group could be the poor metabolizers if the parent compound were the active chemical species and ultrafast metabolizers if the polymorphic pathway produced the active species. Separate analysis would not be necessary for any subgroups that have lower circulating concentrations of the active chemical entity, because they would be at lower risk than the main group in the population.

5) *Additional consideration for* in vitro *studies:*
   - The quality of the samples should be considered and evidence provided that they are representative of the target population (e.g., viability, specific content or activity of marker enzymes).

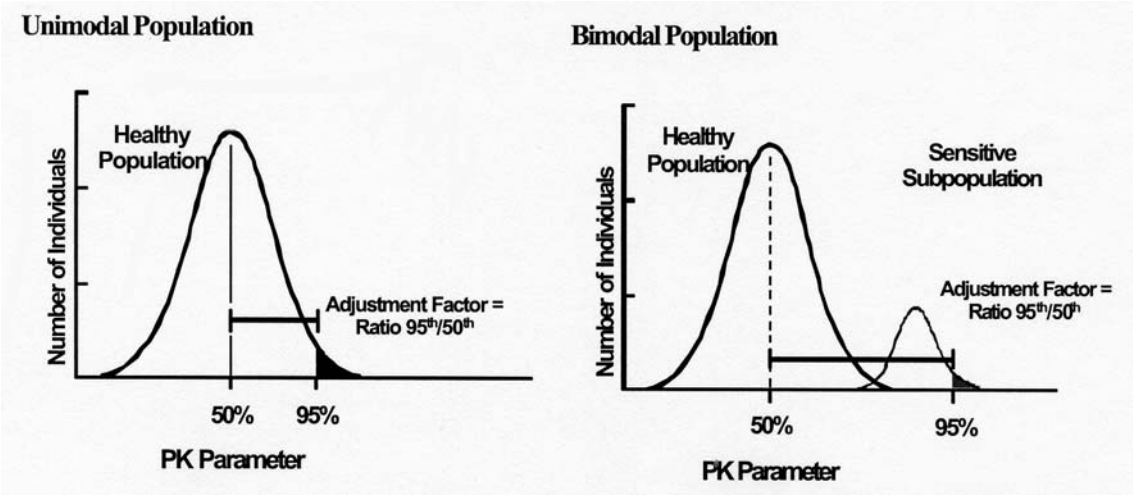


Figure 9. Development of CSAFs for human variability in toxicokinetics/toxicodynamics (HK_{AF}/HD_{AF}). "Healthy population" refers to the general population that is the subject of the risk assessment, which would normally exclude those under medical supervision. PK = Pharmacokinetic.

3.4 Data for the development of a chemical-specific adjustment factor for human variability in toxicodynamics (HD_{AF}) (Figure 10)

While there is a need for adjustment to allow for potentially susceptible subgroups in the human population, in the case where there are adequate data in human populations, these would normally be used as the basis for development of outputs to characterize the dose–response or concentration–response relationship. However, analysis of combined *in vivo* dose/concentration–response data (which reflect toxicokinetics and toxicodynamics) and toxicokinetic data by a kinetic–dynamic link model is relevant to the development of a toxicodynamic adjustment factor (HD_{AF}); this factor could then be modified based on quantitative differences between the subjects in the kinetic–dynamic study and the postulated susceptible subgroup. Data from *in vitro* studies in human tissues are also relevant, but studies published to date have rarely defined the extent of human variability in toxicodynamics (see Case B in Appendix 1). While available data will rarely be sufficient as a basis for developing an HD_{AF}, the relevant considerations are presented below for completeness.

3.4.1 Identification of the active chemical moiety

When applying the scheme to a specific chemical, the first step is to identify the active chemical moiety — i.e., the parent compound or a metabolite that is responsible for the critical effect in question. If the data are not sufficient to draw a conclusion on the toxicologically active moiety, then the conventional default approach should be applied.

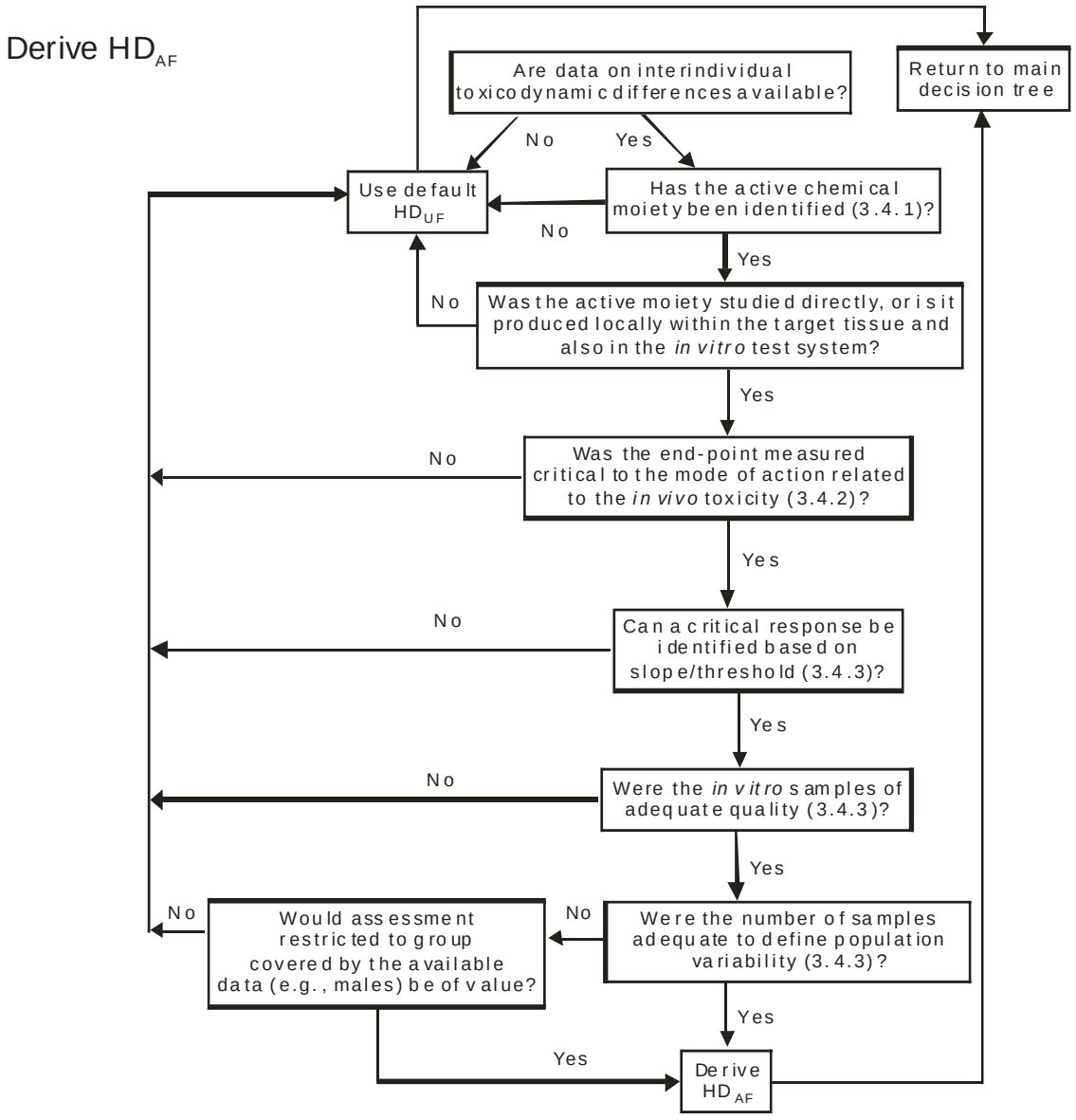


Figure 10. Derivation of HD_{AF} (see accompanying explanatory text).

There are several lines of evidence that may inform the determination of the active chemical moiety:

- The totality of the database on the chemical should be assessed for indications of the role of the parent compound or metabolites in producing the critical toxic effect.
- Data on the mechanism of toxicity of structural analogues may indicate the likely active chemical moiety.

- If there is no metabolism, the critical effect is obviously caused by the parent compound.
- If the chemical is metabolized, then observation of the critical effect after administration of the metabolite(s) may allow identification of the active chemical moiety *in vivo* as well as *in vitro* (see Cases A1 and B in Appendix 1).
- In some cases, there may be data available on the influence of induction or inhibition of metabolism of the chemical on the critical effect. If inhibition (which lowers the clearance of the parent compound and increases its AUC/concentration) is followed by a decrease in the critical effect / decreased effect size, it is likely that the effect is caused by the metabolite. If the effect increases, this is an indication that the active chemical moiety is likely to be the parent compound. The same changes after enzyme induction would lead to the opposite conclusions. The strength of such evidence would be increased by kinetic data demonstrating that the anticipated metabolic pattern occurs *in vivo*.
- The importance of metabolism may be inferred by evaluation of the database on the chemical in relation to differences in dynamic response / critical effect. For example, there may be differences in response in relation to the species and strain studied, the route of administration (e.g., inhalation vs. oral) or the mode of administration (e.g., gavage vs. diet). Such observations should be supported by kinetic data on the parent compound and/or data on metabolism to determine whether the differences in response could be caused by different metabolic patterns; for example, first-pass metabolism could occur after oral but not inhalation administration, while saturation of metabolism is more likely after bolus administration, such as gavage.
- The active chemical moiety must be used in the relevant *in vitro* studies and/or there must be adequate metabolic capacity in the test system for the relevant bioactivation pathway.

3.4.2 Consideration of end-point

The end-point measured should be either the critical effect or a key event. Key events or surrogates are those that are intimately linked to the critical toxic effect based on understanding of mode of action. Dose–response and temporal relationships for key events/surrogates should be consistent with those for the critical toxic effect.

In vitro studies of the toxic response or a surrogate for the toxic end-point in tissues from a wide range of human subjects, or in tissues from average humans and subgroups known to be susceptible to the effect, could provide relevant toxicodynamic data as a basis for development of the HD_{AF}. Such data will define target site sensitivity directly, without any toxicokinetic influences (see Case B in Appendix 1).

3.4.3 Experimental data

Determination of the adequacy of the experimental data as a basis for replacement of the default is made on a case-by-case basis, taking into account a number of aspects of the critical studies, including nature of the population, the concentration–response data and sample size.

1) *Relevance of population:*
   - The humans who were the source of tissue for *in vitro* study should be sufficiently representative of the population at risk of the adverse effect detected in the animal studies (e.g., males for testicular toxicity in Case A in Appendix 1).
   - Ideally, the humans should be of an equivalent age or stage of development to the animals in which the adverse effects were observed; if not, the impact of any discrepancy on the validity of the calculated ratio for susceptible to average humans should be considered.
   - The tissues should be representative of all relevant age groups if there are expected to be variations with age and should include both sexes if there are expected to be variations with sex. In cases where the available *in vitro* data relate to only one population group, the extent to which the tissue sensitivity may differ for other exposed groups should be given careful consideration.

2) *Adequacy of concentration–response data:*
   - Studies must be designed to include a suitable number of concentrations to adequately characterize the concentration–response relationship in humans.
   - Quantitative comparisons of *in vitro* data for development of a human variability toxicodynamic adjustment factor should be based on concentrations that induce an effect of defined magnitude in different human individuals (e.g., EC_{10}). They cannot be based on comparison of responses to a single concentration.
   - The experimental methods measuring the concentration–response relationship in different studies using human tissue should be comparable in order to allow quantitative comparison.
   - Where the concentration–response curves in different individuals/samples are parallel, selection of the point for quantitative comparison (the metric) can be anywhere between 10% and 90% response on the concentration–response curve.
   - Where the curves in different individuals are not parallel, the point for quantitative comparison should be the lowest point on the concentration–response curve that provides reliable information without extrapolation below the experimental data (e.g., EC_{10}).

3) *Adequacy of number of subjects/samples:*
   - The numbers of humans should be sufficient to ensure that the data allow a reliable estimate of the central tendency and the nature of the population distribution for all potentially exposed groups.
   - The distribution of the data should be examined for evidence of any discontinuity, indicating a distinct population subgroup. In practice, the presence of a small number of outliers would not be considered evidence of a discontinuous distribution. Where there is evidence of a discontinuous distribution, the population size should be sufficient to provide information on the nature of the population distribution of both the higher-frequency group and the subgroups.
   - An understanding of the biochemical or physiological basis for the presence of a subgroup (polymorphism) can greatly assist in the design of studies to generate and interpret appropriate data.

- Data on the nature of the population distribution of measurements of the relevant end-point should be used when available.
- In the absence of data on the nature of the population distribution and in the absence of other evidence to the contrary, it should be assumed that the activity of the underlying toxicodynamic process is lognormally distributed within the sample population (because it is a more conservative assumption).
- The central tendency of the selected parameter, such as an EC_{10}, should be estimated as the simple geometric (or arithmetic, if transformed appropriately) mean of the relevant data.
- In general, characterization of variability will relate to the general population (i.e., all age groups). However, where there are potentially susceptible population subgroups, it is recommended that they be addressed separately, with a clear indication of the proportion of the population likely to be susceptible, as a basis for decision-making by risk managers.
- The population distribution should be analysed and the CSAF (HD_{AF}) calculated as the ratio between the central tendency values for the main group and given percentiles (such as 95th, 97.5th and 99th) for the whole population and any potentially susceptible subgroup separately. This is the correct form for the ratio (i.e., average/susceptible), because the EC_{10} for the susceptible subgroup will be lower. Where there are discrete subgroups of the population, the CSAFs for different percentiles should be calculated based on data for the whole population, including the subgroup, and also for the subgroup separately; both sets of results should be provided to the risk manager. Separate analysis would not be necessary for any subgroups that have lower sensitivity to the active chemical entity, because they would be at lower risk than the main group in the population.

4) *Additional considerations for* in vitro *studies:*
   - The quality of the samples should be considered and evidence provided that they are representative of the target population (e.g., viability, specific content or activity of marker enzymes).
   - When limited *in vivo* data in humans are available, although they may be inadequate for direct use in characterization of concentration–response, they can be of value to check that the results of *in vitro* studies used for the development of the HD_{AF} are plausible.

3.5 Incorporation of chemical-specific adjustment factors for interspecies differences and human variability into a composite uncertainty factor

The extent to which the CUF differs from the normal default uncertainty factor of 100 is dependent on the number of adequate and relevant quantitative data available on the chemical. If no such data are available, the CUF will be the normal default value.

The CUF is the product of four different factors, each of which could be a CSAF or a default uncertainty factor:

$$CUF = [AK_{AF} \text{ or } AK_{UF}] \times [AD_{AF} \text{ or } AD_{UF}] \times [HK_{AF} \text{ or } HK_{UF}] \times [HD_{AF} \text{ or } HD_{UF}]$$

Depending on the nature of the data, the CUF can be greater than, less than or even the same as the usual default (normally 100). The CSAF is determined by the chemical-specific data and may be above, below or the same as the default; an interspecies factor could be less than 1 if humans had lower target tissue exposure to the active chemical moiety for the same external dose or showed lower tissue sensitivity. It is important that the entire database is assessed in relation to the possible consequences of the use of a CUF. If the CUF for an effect considered potentially critical based on consideration of the entire database is similar to or exceeds the usual default (e.g., 100), then this concentration/dose–response assessment should be protective for other toxic effects. If, however, the CUF for a potentially critical effect is less than the normal default, a different toxic effect with a higher NOAEL/NOAEC combined with a default uncertainty factor could become the critical effect (see section 2.4).

Recent reviews of risk assessment methods have included the concept of CSAFs (Edler et al., 2002); however, because of the extensive data requirements, there have been only a few examples in which a CUF has been used in risk assessment to date. The concept of sub-dividing the uncertainty factors of 10 has been used in the JECFA evaluations of dioxins (JECFA, 2002) and methylmercury (JECFA, 2004), by JMPR in its evaluation of carbamates (JMPR, 2002), by the Scientific Committee on Food of the European Commission in its evaluation of the sweetener cyclamate (SCF, 2000) and by the US Environmental Protection Agency in its evaluation of boron (US EPA, 2004). JMPR has discussed the appropriate toxicokinetic parameter to use in establishing an acute RfD for compounds the critical effects for which are C_{max} dependent. On the basis that C_{max} varies less than the AUC, JMPR has reduced the uncertainty factor for such pesticides (e.g., carbamates) to 25 (i.e., [2.5 × 2] × [3.12 × 1.61]) from 100, as variability in C_{max} is at least 50% less than for the AUC between both species and individuals (see JMPR, 2002, 2005).

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APPENDIX 1: CASE-STUDIES

The following case-studies are fictional but realistic examples presented to illustrate the principles of development of CSAFs and CUFs. Each case-study presents, where applicable, data on interspecies differences and human variability in toxicokinetics and toxicodynamics for the compound under study. The development of the CSAFs for interspecies differences in toxicokinetics, human variability in toxicokinetics, interspecies differences in toxicodynamics and human variability in toxicodynamics is then described, where relevant. Finally, the calculation of the CUF is shown.

CASE A: Development of AK_{AF} and HK_{AF}

Preamble

Compound A is a low molecular weight, branched-chain, primary aliphatic amine. It has a high solubility in water and most organic solvents (ethanol, acetone) and a pK_a of 10.2; therefore, it is ionized at physiological pH.

Compound A is produced as a microbial breakdown product during sewage treatment and is present at relatively constant low concentrations in drinking-water. The intake of Compound A via drinking-water is estimated to be 0.05 mg/kg body weight per day. *In vitro* studies indicate that it is not readily absorbed across the skin, and exposure models indicate that drinking-water intake is responsible for >99% of total exposure.

There is an extensive and adequate toxicological database on this chemical, with classic metabolism and toxicokinetic studies in humans and test species. The chemical is not genotoxic by any test system. There are adequate subchronic and chronic studies in both rats and mice.

The effect produced by Compound A at the lowest doses (the critical effect) is testicular atrophy in Wistar rats, with a decrease in testes weight at doses of 20 mg/kg body weight per day or more in both subchronic and chronic studies. The lesion is localized germ cell depletion in some seminiferous tubules, with adjacent tubules apparently unaffected. In a multigeneration reproduction study, there was a decrease in male fertility at 20 mg/kg body weight per day and a NOAEL of 10 mg/kg body weight per day.

In contrast to Wistar rats, which excrete about 20% of an oral dose as a hydroxy metabolite (Hydroxy-A), DA rats excreted only about 2% as the hydroxy metabolite. In a 90-day study at 20 mg/kg body weight per day in DA rats, which have only low levels of CYP2D2 (analogous to human CYP2D6), this strain was as sensitive as the Wistar rat to the testicular toxicity of Compound A.

There are no *in vivo* data on the mechanism of action of Compound A on the rat testes. In *in vitro* studies in co-cultures of Sertoli and germ cells from Wistar rats (incubated for 48 h), there was vacuolization in Sertoli cells at concentrations of 0.1 mmol/litre for Compound A and at 1 mmol/litre for Hydroxy-A; Hydroxy-A at 0.1 mmol/litre did not produce detectable

effects *in vitro*. (Note: A Compound A concentration of 0.1 mmol/litre is equivalent to a concentration of approximately 10 µg/ml.) It was concluded that toxicity was produced by a direct effect of the parent compound on the testes.

Interspecies differences in toxicokinetics

Compound A is absorbed completely from the gastrointestinal tract of rats and humans, as determined using ^{14}C-labelled compound. Less than 1% was recovered in the expired air as carbon dioxide, and the urine was the major route of elimination (>95% of dose).

There were clear species differences in urinary metabolites after oral dosing to male rats and humans (Table A-1) (group sizes typically $n = 3$).

Table A-1. Urinary excretion of Compound A and its hydroxy metabolite in male rats and humans.

Species	*Dose (mg/kg body weight)*	*% dose excreted[a] (within 36 h) as*	
		Compound A	*Hydroxy-A*
Rat (Wistar)	1	80 ± 3	20 ± 5
	10	82 ± 5	18 ± 7
	20	80 ± 4	20 ± 3
	40	88 ± 2	12 ± 4
Human	0.1	88 ± 2	0 (not detected)

[a] Mean ± SD.

Plasma toxicokinetic data are available for male rats and male humans after oral dosage. For the rats, the data were derived from concentrations measured in plasma and testes following termination of five animals at different time points after dosage by gavage. The kinetic parameters were calculated using the mean concentration at each time point. Rapid absorption of a single oral dose resulted in peak concentrations of Compound A at 1–3 h after dosage. The kinetic parameters are presented in Table A-2.

In addition to these data for single doses, the plasma and testes AUCs were measured over a 24-h period in rats after 4 weeks of intake of 20 mg/kg body weight per day via the drinking-water. Peak concentrations of Compound A in rats were 0.8 µg/ml and 3.2 µg/g in plasma and testes, respectively; the AUC in plasma (over a 24-h period) was 10 (µg/ml)·h and the plasma clearance (calculated as daily intake/AUC at steady state) was 33 ml/min per kg body weight. The clearance values calculated from this study agreed well with those for the single-dose gavage data.

The ratio of the AUC in testes to the AUC in plasma was 3.8–4.1 in rats, and the time course in testes closely mirrored the time course in plasma.

Table A-2. Plasma toxicokinetic data in rats following a single oral dose.[a]

Species	*Dose (mg/kg body weight)*	*N*	*C_{max} (µg/ml)*	*T_{max} (h)*	*AUC [(µg/ml)·h]*	*Elimination half-life (h)*	*CL (ml/min per kg body weight)*
Rat (Wistar)	1	5	0.49	1	0.46	2	36
	10	5	0.90	1	4.5	2.2	37
	20	5	1.49	3	12.4	2.9	27
	50	5	2.7	4	34.8	3.3	24

[a] Definitions of terms:
N = number of animals studied at each time point (animals were killed and blood and testes were taken for analysis)
C_{max} = maximum observed plasma concentration
T_{max} = time of C_{max}
AUC = area under the plasma concentration–time curve (extrapolated to infinity)
CL = total plasma clearance (calculated assuming bioavailability = 1) (= dose/AUC)

The plasma concentrations of Compound A were determined in a group of 12 healthy adult male human volunteers (aged 20–30 years) given a single oral dose of 0.25 or 1.0 mg/kg body weight in a well conducted randomized cross-over design study (Table A-3).

Table A-3. Plasma and renal toxicokinetic parameters for humans following oral exposure.[a]

Dose (mg/kg body weight)	*N*	*C_{max} (µg/ml)*	*T_{max} (h)*	*Elimination half-life (h)*	*CL[b] (ml/min)*	*Renal CL[b,c] (ml/min)*	*CL[d] (ml/min per kg body weight)*
0.25	12	0.08	1.2	3.5 ± 0.3	805 ± 40	760 ± 35	9.9
1.0	12	0.3	1.2	4.8 ± 0.6	540 ± 50	520 ± 45	6.6

[a] Abbreviations are as in Table A-2 above.
[b] $P < 0.05$ between high and low doses.
[c] Renal CL = renal clearance calculated from amount excreted in urine and plasma concentrations.
[d] The CL adjusted to body weight (ml/min per kg body weight) was not reported, and the values have been calculated as mean CL (ml/min) divided by mean body weight reported in the study.

Compound A is eliminated almost entirely by renal excretion in humans; in rats, elimination is about 80% by renal excretion and 20% by metabolism. The clearance values can be compared with renal physiology (Table A-4). Renal blood flow is relatively constant across species when expressed as ml/min per 100 g kidney tissue (406–632) and as a percentage of cardiac output (13.5–17.5%).

The urine is the major route of elimination of Compound A, and the plasma clearance exceeds the glomerular filtration rate and is equivalent to renal plasma flow in rats and humans. This indicates that the compound undergoes active renal tubular secretion.

Table A-4. Renal physiological parameters in rats and humans.

Species	*Glomerular filtration rate (ml/min per kg body weight)*	*Renal plasma flow (ml/min per kg body weight)*
Rat	6.2	26
Human	1.85	10

Human variability in toxicokinetics

The available data on the toxicokinetics of Compound A in humans are presented above.

Renal plasma flow in humans is measured by the clearance of *para*-aminohippuric acid and is 654 ± 163 ml/min in men and 592 ± 153 ml/min in women (mean ± SD). The glomerular filtration rate in humans is 131 ± 22 ml/min in men and 117 ± 16 ml/min in women. Renal function increases until 20 years of age and then declines at a rate of about 0.8% per annum, so that the glomerular filtration rate in a 90-year-old individual with healthy kidneys would be about 40 ml/min. Ageing is associated with a 30% decrease in renal mass and a 40–50% decline in renal perfusion and renal tubular function. All kinetics data relate to healthy young males (animals and humans), and this was the age group in which testicular toxicity was observed in animal studies.

Interspecies differences and human variability in toxicodynamics

There are no data on the effects of Compound A on human testes (*in vitro* or *in vivo*) or on reproductive capacity in humans. The only data relate to the tolerability of the doses used in the human metabolism/toxicokinetic studies. The maximum single oral dose (1.0 mg/kg body weight) in the volunteers did not produce any adverse effects; this was not a placebo-controlled or double-blind study, and this dose was not used as a NOAEL for risk assessment purposes.

Development of a CSAF for interspecies differences in toxicokinetics (AK_{AF})

Identification of the active chemical moiety

The evidence presented in the preamble from *in vivo* studies in DA rats and from *in vitro* studies indicated that the parent compound was the active entity. All toxicokinetic data were for the active chemical entity.

Choice of relevant toxicokinetic parameter

There are no data to determine whether C_{max} or AUC is the correct toxicokinetic parameter, and therefore the more conservative AUC is used. There was an appropriate study design, and the AUC was extrapolated to infinity, which is necessary for single-dose data (since the AUC to infinity for a single dose equals the AUC for a dose interval at steady state). The AUC and clearance data for a dose interval at steady state in rats supported the single-dose data (but

these data are not essential). For both species, the clearance was calculated after oral dosage with extrapolation to infinity. This is a good measurement of the internal dose, since the oral AUC is related to clearance following oral exposure by the equation AUC = internal dose / CL.

The available data indicate that levels in the testes (the target organ) equilibrate rapidly with the plasma, and the testes are therefore considered as part of the central compartment. The testes:plasma ratio exceeds unity in rats, indicating tissue uptake. A PBPK model would not be useful, unless the tissue uptake in humans could be included based on human data (simply fitting the ratio for rats into a human model would not provide more information than the clearance). Information on the testes:plasma ratios in rats and humans could simply be combined with the corresponding clearances to provide a target organ AUC without the need for a PBPK model.

Experimental data in animals

1) *Relevance of route:*

   The clearance was calculated from oral data and therefore would include any influence of oral bioavailability; this is appropriate, given the route of exposure for humans.

2) *Relevance of dose:*

   The plasma toxicokinetic data in rats were for a range of doses appropriate to the NOAEL. The single-dose gavage studies were supported by the analysis of samples taken during a 4-week drinking-water study. The rat data selected for the development of a CSAF were for the 10 mg/kg body weight dose, as this was the NOAEL.

3) *Adequacy of number of subjects/samples:*

   The data were for five animals at each time point, and the calculated AUC and CL were based on the mean values and represented a good estimate of the central tendency.

Experimental data in humans

1) *Relevance of population:*

   The group studied comprised healthy adult males and therefore was of relevance to the effect of interest (i.e., testicular effects).

2) *Relevance of route:*

   The clearance was calculated from oral data and therefore would include any influence of oral bioavailability; this is appropriate, given the route of exposure for humans.

3) *Relevance of dose:*

The data for doses of 0.25 and 1.0 mg/kg body weight indicated the possibility of saturation kinetics in humans. The dose of 0.25 mg/kg body weight was selected, since the higher dose represents 10% of the animal NOAEL, and tolerable human exposures would not reach this level (see below).

4) *Adequacy of number of subjects/samples:*

The plasma toxicokinetic data in humans were for a sufficient number of individuals, in relation to the very low standard deviation in the data, to give an adequate estimate of the central tendency. The standard error (i.e., standard deviation divided by the square root of the sample number: $40/12^{0.5} = 11.5$) is only 1.4% of the mean (805) and is therefore acceptable.

Calculation of a CSAF for interspecies differences in toxicokinetics (AK_{AF})

The clearance in rats (37 ml/min per kg body weight at 10 mg/kg body weight) was divided by the clearance in humans (9.9 ml/min per kg body weight at 0.25 mg/kg body weight), since this would represent the ratio by which the human internal dose would be greater than the rat internal dose.

The CSAF (AK_{AF}) is 3.7. This value is very close to the default, as would be expected, given the basis for the selection of the default values. The AK_{AF} should be used rather than the default because it is based on chemical-specific data.

Development of a CSAF for human variability in toxicokinetics (HK_{AF})

Identification of the active chemical moiety

The evidence given in the preamble from *in vivo* studies in DA rats and from *in vitro* studies indicated that the parent compound was the active entity. All toxicokinetic data were for the active chemical entity.

Choice of relevant toxicokinetic parameter

The plasma toxicokinetic data in humans were for 12 individuals aged 20–30 years, which is adequate to define the central tendency, but inadequate to define the potential variability in the human population. The human data (supported by the animal data) were adequate to establish that renal tubular secretion was the major elimination pathway; given the essentially complete absorption, renal blood flow is the critical physiological variable determining the oral AUC and the variability between individuals.

Experimental data

The major determinant of interindividual variability will be differences in renal blood flow, for which there are robust data, and this physiological variable can be used to derive an HK_{AF}. Because the adverse effect is on the testes, the renal blood flow data for men (654 ± 163 ml/min; data compiled from physiology literature sources) were used as the basis for the calculation. The interindividual variability in human data (Table A-3) was limited compared with known variability in renal blood flow and glomerular filtration rate, and, therefore, the data were not considered to reflect the full range of human variability.

Calculation of a CSAF for human variability in toxicokinetics (HK_{AF})

The renal blood flow 2 standard deviations (326 ml/min) below the mean (654 ml/min) is 328 ml/min, and therefore the HK_{AF} would be 654/328 (= 1.99), because the lower renal blood flow would give a higher AUC. The CSAF (HK_{AF}) is therefore 2.0. It should be noted that the use of 2 standard deviations below the mean is only one approach that can be used in this calculation. Alternatively, the assessment could provide a range of outputs for different percentiles of the population based on the mean renal blood flow (654 ml/min), its standard deviation (163 ml/min) and the assumption that the population distribution of renal blood flow is normally or lognormally distributed. Assuming a normal distribution, the HK_{AF}s necessary to allow for the difference between the mean and the 95th, 97.5th and 99th percentiles of the population are 1.7, 2.0 and 2.4 using the method outlined in the second bullet point below (these represent the factors by which the mean renal blood flow would have to be divided to give a renal blood flow equivalent for the percentile of the population).

Notes:

- Because a normal distribution is assumed, the resulting factor is dependent on the direction of the difference; for example, the ratio for 2 standard deviations above the mean would be 1.5 (980/654). The use of a lognormal distribution has the advantage that it gives the same ratios for *n* standard deviations above and below the mean.
- The factors for different percentiles of the population assuming a normal distribution can be calculated using NORMDIST on Excel using the formula: distribution = NORMDIST((654/factor),654,163,true), with the factor varied to give distributions of 0.05, 0.025 and 0.01 (equivalent to 95th, 97.5th and 99th percentiles).
- To non-statisticians, there may appear to be a "discrepancy" between the factor derived by applying 2 standard deviations to the mean (2.0) (it is generally known that 2 standard deviations cover 95% of the population) and the factor calculated by distribution analysis to cover 95% of the population (1.7). The reason for this is that 2 standard deviations around the mean include 95% of the total population, with 2.5% of the population in each tail of the symmetrical distribution. A factor of 2.0 is obtained for the 97.5th percentile from the NORMDIST analysis.
- The factors for different percentiles of the population assuming a lognormal distribution can be calculated using NORMSINV on Excel. The NORMSINV values for the 95th, 97.5th and 99th percentiles of a lognormal distribution are 1.64, 1.96 and 2.33, respectively. The ratios between the geometric mean estimate and the estimate value at these

percentiles are given by the antilog of the NORMSINV value multiplied by the log of the geometric standard deviation (log GSD).

- It is important that this form of dose/concentration–response assessment is explained clearly, because both normal and lognormal distributions reach zero on the y-axis only at zero and infinity on the x-axis — i.e., the factor necessary to cover everyone would be infinity.

Consideration of susceptible subgroups

There are no chemical-specific data on the clearance of Compound A in children; however, their renal blood flow is higher that that of adults. This would give a lower AUC in children than in adults receiving the same intake on a mg/kg body weight basis, and therefore the value of HK_{AF} would cover this group as well.

Calculation of the composite uncertainty factor (CUF)

The total factor is a composite of adjustment factors for interspecies differences and human variability in toxicokinetics and uncertainty factors for interspecies differences and human variability in toxicodynamics. The CUF is generally shown as:

$$CUF = [AK_{AF} \text{ or } AK_{UF}] \times [AD_{AF} \text{ or } AD_{UF}] \times [HK_{AF} \text{ or } HK_{UF}] \times [HD_{AF} \text{ or } HD_{UF}]$$

In this case:

$$CUF = AK_{AF} \times AD_{UF} \times HK_{AF} \times HD_{UF}$$

The output will depend on the percentile of the population to be covered by the CUF:

- for 95th percentile: $3.7 \times 2.5 \times 1.7 \times 3.16 = 49.69 = 50$
- for 97.5th percentile: $3.7 \times 2.5 \times 2.0 \times 3.16 = 58.46 = 60$
- for 99th percentile: $3.7 \times 2.5 \times 2.4 \times 3.16 = 70.15 = 70$

Dividing the NOAEL of 10 mg/kg body weight per day by a factor of 50–70 results in intakes of 0.14–0.20 mg/kg body weight, and therefore the dose of 0.25 mg/kg body weight that was selected for calculating the AK_{AF} was appropriate.

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CASE A1: Development of AK_{AF} and HK_{AF}

Preamble

Compound A1 is a weak basic alicyclic aliphatic hydroxylamine (pK_a 8) that is readily soluble in organic solvents but is sparingly soluble in water. The exposure scenario is identical to that for Compound A.

This is similar to Case A but raises different issues for consideration. The database is identical in relation to:

- data on toxicity and the critical effect (testicular toxicity)
- the most sensitive test species (rats)
- the exposure scenario
- the dose–response and NOAEL (10 mg/kg body weight per day) in the test species.

There are no relevant data on mode of action. There are no *in vitro* studies in rat testicular cultures.

Compound A1 is oxidized to an alicyclic alcohol by liver microsomes and is dependent on NADPH and inhibited by carbon monoxide (i.e., it is probably catalysed by cytochrome P450), but there are no modern data relating to the form of P450 responsible.

The only data in humans relate to the single-dose ADME and toxicokinetic studies, in which Compound A1 was well tolerated without adverse health effects.

The alicyclic alcohol metabolite did not produce testicular effects when given at doses of up to 100 mg/kg body weight per day for 90 days.

Interspecies differences in toxicokinetics

The compound is well absorbed from the gut in all species and eliminated in urine (>95% of the dose) as the "deaminated" alicyclic alcohol and its glucuronide, with negligible excretion of the parent compound or other metabolites (Table A-5).

Table A-5. Urinary excretion of Compound A1 and its metabolites.

Species	*Dose (mg/kg body weight)*	*N*	*% dose excreted[a] in urine (15 days total) as*		
			Alicyclic alcohol	*Glucuronide of alicyclic alcohol*	*Compound A1*
Rat	10	5	17	82	1
Human	0.1	6	1 ± 1	96 ± 2	3 ± 1

[a] Mean for rats; mean ± SD for humans.

Plasma toxicokinetic data on the parent hydroxylamine are available for male rats and humans (Table A-6).

The data for male rats were determined by serial termination of five animals at each time point and using the mean data to calculate the kinetic parameters. The data for humans relate to 12 healthy adult males aged 20–30 years.

Table A-6. Plasma toxicokinetic data in male rats and humans.[a]

Species	*Dose (mg/kg body weight)*	*N*	*C_{max} (µg/ml)*	*T_{max} (h)*	*AUC [(µg/ml)·h]*	*Elimination half-life (h)*	*CL (ml/min per kg body weight)*
Rat	20	5	4.8	1	30	4	11
Human	0.25	12	0.03 ± 0.02	3	8 ± 12	80 ± 120	0.5 ± 0.8

[a] Abbreviations are as in Table A-2 above.

Human variability in toxicokinetics

The available data on the toxicokinetics of Compound A1 are presented in Table A-6 above.

Development of a CSAF for interspecies differences in toxicokinetics (AK_{AF})

Identification of the active chemical moiety

The evidence presented in the preamble indicates that the alcohol metabolite did not produce testicular toxicity at doses 10 times the NOAEL of the parent compound, and therefore the metabolite can be considered to be inactive. It is considered that the glucuronic acid conjugate is an inactive detoxication product. All toxicokinetic data were for the active chemical entity.

Choice of relevant toxicokinetic parameter

There are no data to determine whether C_{max} or AUC is the correct toxicokinetic parameter, and therefore the more conservative AUC is used. There was an appropriate study design, and the AUC was extrapolated to infinity, which is necessary for single-dose data (since the AUC to infinity for a single dose equals the AUC for a dose interval at steady state). For both species, the clearance was calculated after oral dosage with extrapolation to infinity. This is a good measurement of the internal dose, since the oral AUC is related to clearance following oral exposure by the equation AUC = internal dose / CL.

Experimental data in animals

1) *Relevance of route:*

   The clearance was calculated from oral data and therefore would include any influence of oral bioavailability; this is appropriate, given the route of exposure for humans.

2) *Relevance of dose:*

   The plasma toxicokinetic data in rats were for a dose close to the NOAEL. The dose is above the NOAEL, and if saturation of metabolism were to occur between the NOAEL

and the dose used in the kinetic studies, it would tend to increase the AUC of the parent compound and therefore reduce the difference between rats and humans.

3) *Adequacy of number of subjects/samples:*

The data were for five animals at each time point, and the calculated AUC and clearance were based on the mean values and represented a good estimate of the central tendency.

Experimental data in humans

1) *Relevance of population:*

The group studied comprised healthy adult males and was therefore of relevance to the critical effect (i.e., testicular toxicity).

2) *Relevance of route:*

The clearance was calculated from oral data and therefore would include any influence of oral bioavailability; this is appropriate, given the route of exposure for humans.

3) *Relevance of dose:*

The dose studied was 0.25 mg/kg body weight, which represents 2.5% of the animal NOAEL and would be appropriate for an initial assessment.

4) *Adequacy of number of subjects/samples:*

The plasma toxicokinetic data in humans were for 12 individuals and showed very large variability in clearance. The standard error (i.e., standard deviation divided by the square root of the sample number: $0.8/12^{0.5} = 0.23$) is 46% of the mean (0.5), which is quite large. Such variability is possible when a compound is eliminated by a metabolic pathway that is subject to genetic polymorphism, but high variability could also arise from inadequate data (this possibility would need to be resolved before determining a CSAF). In this case, the variability is from a well designed and performed study, and the pathway of elimination is probably via cytochrome P450, for which there is genetic polymorphism for some forms. The number of subjects is inadequate to define the population distribution of the clearance of the compound or the central tendency of the data with sufficient confidence.

Calculation of a CSAF for interspecies differences in toxicokinetics (AK_{AF})

An analysis of the population distribution would be necessary as a basis for development of the CSAF for interspecies differences in toxicokinetics (AK_{AF}). The number of subjects for which there are data is inadequate to be used to develop an AK_{AF}. (However, if there is already human exposure and risk managers consider that risk characterization is essential, then advice could be given that the substantial species difference in clearance should not be

ignored. Under such circumstances, an interim factor of 22 [which would be too insecure to be termed an AK_{AF}] could be based on the ratio of the clearance values [11/0.5].)

Development of a CSAF for human variability in toxicokinetics (HK_{AF})

Identification of the active chemical moiety

The evidence given in the preamble states that the alcohol metabolite did not produce testicular toxicity at doses 10 times the NOAEL of the parent compound, and therefore the metabolite can be considered to be inactive. All toxicokinetic data were for the active chemical entity.

Choice of relevant toxicokinetic parameter

There are no data to determine whether C_{max} or AUC is the correct toxicokinetic parameter, and therefore the more conservative AUC is used. There was an appropriate study design, and the AUC was extrapolated to infinity, which is necessary for single-dose data (since the AUC to infinity for a single dose equals the AUC for a dose interval at steady state). The clearance was calculated after oral dosage with extrapolation to infinity. This is a good measurement of the internal dose, since the oral AUC is related to clearance following oral exposure by the equation AUC = internal dose / CL.

Experimental data

The plasma toxicokinetic data in humans were for 12 individuals aged 20–30 years, which is inadequate to define the potential variability in the human population. An analysis of the population distribution of clearance in a relatively large population (depending on the frequency of any polymorphism) would be necessary. In addition, it would be valuable to know the basis of the polymorphism.

Calculation of a CSAF for human variability in toxicokinetics (HK_{AF})

An analysis of the population distribution would be necessary as a basis for development of the CSAF for human variability in toxicokinetics (HK_{AF}). The number of subjects for which there are data is inadequate as a basis for development of an HK_{AF}. If adequate data were available that indicated that the *in vivo* variability arose from, for example, CYP2D6, which has an incidence of about 8% poor metabolizers, then the adjustment factor would be based on the ratio of the mean *in vivo* parameter estimate of the extensive metabolizers to the 95th percentile of the *in vivo* parameter estimate for poor metabolizers using chemical-specific data. (However, if there is already human exposure and risk managers consider that risk characterization is essential, then advice could be given that the wide interindividual differences in clearance should not be ignored. Under such circumstances, an interim factor [which would be much too insecure to be termed an HK_{AF}] could be based on the available data on variability. In addition, the calculated ratio could be adjusted by known differences between extensive and poor metabolizers for other substrates of the polymorphic enzyme [see Dorne et al., 2002].)

Calculation of the composite uncertainty factor (CUF)

The available data indicate that the use of the default uncertainty factors (i.e., 10 × 10) would be inappropriate, but the database was inadequate for the development of CSAFs with confidence. In consequence, a factor (suitably modified away from the default) would be developed only if essential and would not be a CSAF.

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CASE A2: Development of AK_{AF} and HK_{AF}

Preamble

Compound A2 is a weak basic alicyclic aliphatic hydroxylamine (pK_a 8) that is readily soluble in organic solvents but sparingly soluble in water. The exposure scenario is identical to that of Case A.

This is similar to Case A but raises different issues for consideration. The database is identical in relation to:

- the critical effect (testicular toxicity) and toxicity data
- the most sensitive test species (rats)
- the exposure scenario
- the dose–response and NOAEL (10 mg/kg body weight per day) in the test species.

There are no relevant data on mode of action. There are no *in vitro* studies in rat testicular cultures.

Interspecies differences in toxicokinetics

Compound A2 is absorbed completely from the gut in all species. It is eliminated in the urine (>95% of the dose) as the parent hydroxylamine and its glucuronide. The metabolism is similar in all species, with 5–10% excreted as Compound A2 and the remainder as the glucuronide. The toxicokinetics of the parent compound have been studied in male rats and male humans (Table A-7).

Table A-7. Plasma toxicokinetic data for Compound A-2 in male rats and humans.[a,b]

Species	*Dose (mg/kg body weight)*	*N*	*C_{max} (µg/ml)*	*T_{max} (h)*	*AUC [(µg/ml)·h]*	*Elimination half-life (h)*	*CL (ml/min per kg body weight)*
Rat	20	5	4.8	1	30	4	11
Human	0.25	12	0.06 ± 0.02	1	0.14 ± 0.04	1.5 ± 0.5	30 ± 9

[a] Abbreviations are as in Table A-2 above.
[b] Mean for rats, mean ± SD for humans.

The data for male rats were determined by serial termination of five animals at each time point, with the mean data used to calculate the kinetic parameters. The human data are for 12 healthy young adult males aged 20–30 years. The conjugation is performed primarily in the intestinal wall and liver. It is possible that the compound undergoes first-pass metabolism by conjugation with glucuronic acid in the intestinal wall, and this may be the reason for the species differences in AUC and clearance following oral exposure. There are no kinetics data in rats or humans following a parenteral dose, because all human exposure is believed to occur via the oral route.

Human variability in toxicokinetics

The available data on the toxicokinetics of Compound A2 are presented in Table A-7 above.

Development of a CSAF for interspecies differences in toxicokinetics

Identification of the active chemical moiety

There are no data on the activity of the glucuronide conjugate; however, such glucuronidation probably represents a major detoxication pathway, and therefore the parent hydroxylamine is considered to be the active moiety for the testicular toxicity detected in animals.

Choice of relevant toxicokinetic parameter

There are no data to determine whether C_{max} or AUC is the correct toxicokinetic parameter, and therefore the more conservative AUC is used. There was an appropriate study design, and the AUC was extrapolated to infinity, which is necessary for single-dose data (since the AUC to infinity for a single dose equals the AUC for a dose interval at steady state). For both species, the clearance was calculated after oral dosage with extrapolation to infinity. This is a good measurement of the internal dose, since the oral AUC is related to clearance following oral exposure by the equation AUC = internal dose / CL.

Experimental data in animals

1) *Relevance of route:*

   The clearance was calculated from oral data and therefore would include any influence of oral bioavailability; this is appropriate, given the route of exposure for humans.

2) *Relevance of dose:*

   The plasma toxicokinetic data in rats were for a dose close to the NOAEL. The dose is above the NOAEL; if saturation of metabolism were to occur between the NOAEL and the dose used in the kinetic studies, it would tend to increase the AUC of the parent compound and therefore reduce the difference between rats and humans.

3) *Adequacy of number of subjects/samples:*

The data were for five animals at each time point, and the calculated AUC and clearance were based on the mean values and represented a good estimate of the central tendency.

Experimental data in humans

1) *Relevance of population:*

The group studied comprised healthy adult males and is, therefore, of relevance to the risk assessment for the relevant effect (testicular toxicity).

2) *Relevance of route:*

The clearance was calculated from oral data and therefore would include any influence of oral bioavailability; this is appropriate, given the route of exposure for humans.

3) *Relevance of dose:*

The dose of 0.25 mg/kg body weight represents 2.5% of the animal NOAEL, and tolerable human exposures would not reach this level (unless the total uncertainty factor was less than 40; see below).

4) *Adequacy of number of subjects/samples:*

The plasma toxicokinetic data in humans were for 12 individuals, indicated only limited variability and are adequate to estimate the central tendency. The standard error (i.e., standard deviation divided by the square root of the sample number: $9/12^{0.5} = 2.5$) is only 9% of the mean (30) and is adequate for deriving an AK_{AF}.

Calculation of a CSAF for interspecies differences in toxicokinetics (AK_{AF})

The clearance in rats (11 ml/min per kg body weight at 20 mg/kg body weight) was divided by the clearance in humans (30 ml/min per kg body weight at 0.25 mg/kg body weight), since this would represent the ratio by which the human internal dose would be lower than the rat internal dose. The CSAF (AK_{AF}) is 0.37, i.e., there would be lower concentrations in human blood than in rat blood for the same daily intake per kg body weight because of the higher clearance.

Development of a CSAF for human variability in toxicokinetics (HK_{AF})

Identification of the active chemical moiety

There are no data on the activity of the glucuronide conjugate; however, glucuronidation represents a major detoxication pathway, and therefore the parent hydroxylamine is considered to be the active moiety for the testicular toxicity observed in animals.

Choice of relevant toxicokinetic parameter

There are no data to determine whether C_{max} or AUC is the correct toxicokinetic parameter, and therefore the more conservative AUC is used. There was an appropriate study design, and the AUC was extrapolated to infinity, which is necessary for single-dose data (since the AUC to infinity for a single dose equals the AUC for a dose interval at steady state). The clearance was calculated after oral dosage with extrapolation to infinity. This is a good measurement of the internal dose, since the oral AUC is related to clearance following oral exposure by the equation AUC = internal dose / CL.

Experimental data

The group size and range of subjects studied were considered inadequate to define human variability in the clearance of this compound.

Calculation of a CSAF for human variability in toxicokinetics (HK_{AF})

A CSAF could not be developed based on the chemical-specific data. (A factor related to, but not specific for, Compound A2 could be derived from a review of databases for other compounds undergoing complete absorption and elimination by glucuronidation, providing that the form of the transferase was known.)

Consideration of susceptible subgroups

There are no chemical-specific data on the clearance of Compound A2 in children; however, clearance of drugs by glucuronidation is higher in children than in adults. This would give a lower AUC in children than in adults receiving the same intake on a mg/kg body weight basis.

Calculation of the composite uncertainty factor (CUF)

The CUF is a composite of the adjustment factor for interspecies differences in toxicokinetics and default uncertainty factors for human variability in toxicokinetics and for interspecies differences and human variability in toxicodynamics. The CUF is generally shown as:

$$CUF = [AK_{AF} \text{ or } AK_{UF}] \times [AD_{AF} \text{ or } AD_{UF}] \times [HK_{AF} \text{ or } HK_{UF}] \times [HD_{AF} \text{ or } HD_{UF}]$$

In this case:

$$CUF = AK_{AF} \times AD_{UF} \times HK_{UF} \times HD_{UF}$$

Therefore, CUF = 0.37 × 2.5 × 3.16 × 3.16 = 9. This low uncertainty factor needs consideration in relation to two aspects:

1. Application of the total factor of 9 to the NOAEL for testicular toxicity in rats (10 mg/kg body weight) would give a TDI (or RfD) of 1.1 mg/kg body weight. The human

toxicokinetic data were for a dose of 0.25 mg/kg body weight, which is below the possible TDI. The possibility of non-linear kinetics would need to be considered. In this case, it is very unlikely that the process of glucuronidation would be saturated at a dose of 1.1 mg/kg body weight per day, and therefore the available kinetics data are appropriate for this higher dose.

2. The total factor of 9 means that a different toxic end-point, observed in animal studies at higher doses but with the default 100-fold factor, may become the critical effect. As indicated in IPCS (1994) and above in this document (section 2.4), it is essential under such circumstances to go back to the top of the decision tree and consider other end-points observed at higher doses. Given the simple metabolism of this compound, it is likely that a total uncertainty factor of 9 would be applicable to most other end-points, but each of these should be considered in detail. (For example, adverse effects detected in the urinary bladder might arise from local hydrolysis of the excreted glucuronide, so that the usual 100-fold default uncertainty factor, including the default for interspecies differences in toxicokinetics, would be appropriate.)

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CASE B: Development of AK_{AF}, AD_{AF}, HK_{AF} and HD_{AF}

Preamble

The principal route of exposure of the general population to Compound B is inhalation.

The critical effects of Compound B are those on the haematological system; indeed, these are the effects observed at the lowest concentrations in all species exposed by all routes of administration in both short- and long-term studies. Specifically, exposure results in alterations in haematological parameters characteristic of haemolytic anaemia, haemoglobinuria or increased osmotic fragility of erythrocytes.

The specific mode of action by which Compound B induces haematological effects has not been established. Based on their progression, changes, which include erythrocyte swelling, morphological changes and decreased deformability, are likely due to conjugation of the active acid metabolite of Compound B with the lipids in the membrane of erythrocytes and resulting increases in permeability to cations and water. Data suggest that older red blood cells are more susceptible to Compound B; as they are replaced by more resilient younger cells, the severity of the haematotoxic response declines.

In the primary pathway of metabolism, Compound B is first oxidized in the liver via alcohol dehydrogenase to the intermediate aldehyde, which is subsequently further oxidized via aldehyde dehydrogenase to the corresponding acetic acid derivative, which is conjugated with glycine or glutamine or eventually metabolized to carbon dioxide.

Observed variations in toxicity with sex, age, duration and species correlated well with differences in production and clearance of the acetic acid metabolite. These observations and

additional studies in which oxidation of Compound B to the corresponding acid was inhibited indicate that the acid metabolite is principally responsible for the haematological effects observed in experimental animals exposed to the compound.

BMC_{05}s (benchmark concentrations for a 5% response) for haematological effects range from 5.3 to 61 mg/m^3 in rats and from 10 to 115 mg/m^3 in mice.[1] In rats, the most sensitive end-point, based solely on these estimates of potency, is an increase in mean red cell haemoglobin; in mice, the lowest BMC_{05} was that derived for increased platelets. In general, the BMC_{05}s for each parameter are lower for rats than for mice. Similarly, BMC_{05}s were generally lower in female rats than in male rats, which correlates with observed differences between females and males in clearance of the putative active acid metabolite, along with the fact that significant changes appeared earlier in females than in males.

Interspecies differences and human variability in toxicokinetics

The toxicokinetics of Compound B in groups of 16 male and 16 female F344 rats (the same strain as that used in the critical investigation on which the effect level or BMC is based) exposed to 151, 303 or 605 mg/m^3 and 303, 605 or 1210 mg/m^3 6 h/day, 5 days/week, respectively, for up to 18 months have been investigated. Post-exposure blood samples were collected at 1 day, 2 weeks and 3, 6, 12 and 18 months; post-exposure 16-h urine samples were collected at 2 weeks and 3, 6, 12 and 18 months of exposure. In rats, females were less efficient than males at clearing the acetic acid metabolite from the blood (e.g., half-lives of 64 vs. 40 min at 303 mg/m^3 for 1 day), which appeared to be related to slower renal excretion.

The mean AUC value for male and female rats exposed for various periods in this study (6 h/day for up to 18 months) for the post-exposure period was 999 (µmol/litre)·h (value for 151 mg/m^3).

There are some limited toxicokinetic data available from studies in humans. In an experimental study, five male subjects were exposed by inhalation to Compound B at 97 mg/m^3 during a 2-h period of light physical exercise (50 W), and the acetic acid metabolite was determined in venous blood samples at 0, 2, 4 and 6 h from the start of exposure. Concentrations of the acetic acid metabolite peaked after 2–4 h; in three subjects, peak concentrations were 36–46 µmol/litre (average 41) after 2 h, and in two subjects, peak concentrations were 52–60 µmol/litre (average 56) after 4 h. The average half-life was 4.3 h (range 1.7–9.6 h). The tabulated mean data were analysed using a non-compartmental model and a standard kinetic program (WinNonLin), which gave an AUC of 230 (µmol/litre)·h to 7.1 h, a terminal half-life of 4.5 h and an AUC extrapolated to infinity of 414 (µmol/litre)·h. (The AUC for a single dose extrapolated to infinity equals the AUC at steady state, assuming that there is no change in formation or clearance during chronic intake.)

PBPK models have been developed for the uptake and metabolism of Compound B and the circulating concentrations and renal excretion of its acetic acid metabolite. Scaling of the models to humans is largely based on adjustment by (body weight)$^{0.7}$ of metabolic parameters

[1] Conversion factor in air for Compound B: 1 ppm = 4.84 mg/m^3.

plus appropriate physiologically based differences in organ weights and organ blood flow. These scaled parameter estimates and the model for humans were validated by comparison with the limited published data on the concentrations of Compound B in blood and excretion of its acetic acid metabolite in humans, for which the model provides reasonable fits.

In the first model, compartments included lung, rapidly and slowly perfused tissues, fat, skin, muscle, gastrointestinal tract and liver for both Compound B and its acetic acid metabolite; the kidney was also added to the description for the acetic acid metabolite. Partition coefficients were determined experimentally for both parent compound and the acetic acid metabolite. Rate constants were either taken from the literature (V_{max} for parent to acetic acid metabolite) or estimated by fitting simulations to experimental data (V_{max} for Compound B to other metabolites and renal elimination constants for acetic acid metabolite). In the absence of data, constants for binding to blood protein for the acetic acid metabolite were set to improve the fit of the model. The model reasonably simulated data from studies in young and old male rats exposed intravenously to both parent compound and acetic acid metabolite, with no adjustment of any parameters. Fits of simulations in the case of older rats were improved slightly by increasing the volume of the fat compartment and by decreasing the renal elimination rate, both of which were rationalized as having a biological basis (i.e., these are common changes in ageing rats).

The model also gave reasonable predictions of urinary acetic acid metabolite levels, especially at doses below those demonstrated to cause haemolysis. The model predicted uptake and metabolism of parent compound and elimination of acetic acid metabolite in an inhalation experiment in male rats except at the highest concentration, where the amount of acetic acid metabolite excreted was overpredicted. This was possibly due to toxicity as manifested by haemolysis.

The second model to describe the toxicokinetics of parent compound and acetic acid metabolite in different species following repeated long-term exposures was developed for the most part using 2-week exposures of male rats to Compound B. Adjustments were made to account for differences in female rats, and then further adjustments were made to better simulate data in mice. The model was subsequently expanded to account for age-related differences likely to be evident in the chronic studies used to develop the model.

Structural differences in the second model included separate kidney and spleen compartments and the inclusion of the muscle in the slowly perfused tissue compartment. Tissue:blood partition coefficients were the same as those in the first model, and organ weights as percentages of body weight were very similar in the two models, with the exception of those for liver and lung.

Interspecies differences in toxicodynamics

The effects of the acetic acid metabolite of Compound B on erythrocytes from rats and humans have been examined *in vitro*. Pooled erythrocytes from 9- to 13-week-old F344 rats were exposed to the acetic acid metabolite of Compound B. Erythrocytes were also obtained from healthy human volunteers (men and women, 18–40 years old; n = 5) and similarly

exposed to the acetic acid metabolite. At the end of the incubation period (0.25–4 h), haematocrit and free plasma haemoglobin levels were determined as indicators of swelling of the erythrocytes and haemolysis, respectively. Haemolysis was induced in rat blood by the acetic acid metabolite of Compound B. In contrast, human cells were relatively resistant to the haemolytic effects of the acetic acid metabolite up to the concentrations tested. The data for rats and humans are presented in Tables A-8 and A-9 below, respectively.

Table A-8. Effects of concentration and time on haematocrit and the concentration of free plasma haemoglobin in rat blood incubated with the acetic acid metabolite of Compound B *in vitro*.[a]

	Time since exposure (h)				
	0.25	***0.5***	***1***	***2***	***4[b]***
Haematocrit (% control)					
0.5 mmol/litre	–	104 ± 2	108 ± 2	110 ± 2	121* ± 2
1.0 mmol/litre	–	111 ± 1	117 ± 1	124 ± 2	144* ± 2
2.0 mmol/litre	111 ± 3	118 ± 1	133 ± 2	169 ± 3	170* ± 3
Plasma haemoglobin (g/dl)					
0.5 mmol/litre	–	0.2 ± 0.05	0.2 ± 0.05	0.2 ± 0.05	0.5 ± 1
1.0 mmol/litre	–	0.4 ± 0.05	0.8 ± 0.05	1 ± 0.05	2 ± 1
2.0 mmol/litre	–	0.6 ± 0.05	1 ± 0.05	2.2 ± 1	7* ± 1

[a] Values are the mean ± SD of six determinations (three males, three females). The concentration of free plasma haemoglobin in the plasma of control rats was 0.1–0.2 g/dl.
[b] Asterisk (*) indicates a statistically significant increase compared with controls.

Table A-9. Effects of concentration, time and sex on haematocrit and the concentration of free plasma haemoglobin in human blood incubated with the acetic acid metabolite of Compound B *in vitro*.[a]

	Time since exposure (h)					
	Males			***Females***		
	1	***2***	***4***	***1***	***2***	***4***
Haematocrit (% control)						
2.0 mmol/litre	101 ± 2	103 ± 2	103 ± 2	99 ± 3	100 ± 1	100 ± 3
4.0 mmol/litre	102 ± 1	103 ± 3	105 ± 3	100 ± 2	101 ± 2	103 ± 2
8.0 mmol/litre	104 ± 3	105 ± 1	108 ± 4	104 ± 1	104 ± 3	106 ± 1
Plasma haemoglobin (g/dl)						
2.0 mmol/litre	0.12 ± 0.05	0.13 ± 0.1	0.2 ± 0.05	0.14 ± 0.05	0.15 ± 0.05	0.17 ± 0.05
4.0 mmol/litre	0.17 ± 0.05	0.22 ± 0.05	0.3 ± 0.1	0.2 ± 0.05	0.25 ± 0.05	0.25 ± 0.05
8.0 mmol/litre	0.4 ± 0.05	0.42 ± 0.1	0.53 ± 0.1	0.35 ± 0.05	0.39 ± 0.1	0.44 ± 0.1

[a] Values are the mean ± SD of five determinations.

These data are consistent with the results of several other studies. For example, haemolysis in blood from four adult male Wistar rats and human erythrocytes isolated from the blood of healthy adult male donors (no further details provided) was examined *in vitro*. The lowest concentration of acetic acid metabolite administered (1.25 mmol/litre) resulted in 25% haemolysis of rat erythrocytes after 180 min. In contrast, an acetic acid metabolite concentration of 15 mmol/litre did not produce measurable haemolysis in human erythrocytes over the same time. This study was conducted in washed erythrocytes rather than whole blood. In another study, the maximum concentration (2 mmol/litre) did not produce any detectable effect in human erythrocytes, although it induced rapid haemolysis in rat erythrocytes. Exposure of rat erythrocytes to the acetic acid metabolite at a concentration of 0.2 mmol/litre did not result in haemolysis, although reduced cell deformability and increased mean cell volume were noted.

Human variability in toxicodynamics

A further study was conducted in which erythrocytes from various human sources were investigated for their response to the acetic acid metabolite of Compound B. Cells were obtained from 18 healthy individuals, 9 with a mean age of 41 years (range 31–56 years; 5 men, 4 women) and 9 with a mean age of 72 years (range 64–79 years; 5 men, 4 women), from 7 people with sickle cell disease and from 3 people with spherocytosis. The haemolytic activity, assessed as percent haemolysis of cells, of 2 mmol/litre of the acetic acid derivative of Compound B was tested on the purified erythrocytes from these groups by incubating the cells with the acetic acid derivative for up to 4 h. The results obtained are given in Table A-10.

Table A-10. Percent haemolysis in human erythrocytes exposed *in vitro* to 2 mmol/litre of the acetic acid metabolite of Compound B.[a]

Source of cells	*N*	*% haemolysis after following treatment:*					
		0 h		*2 h*		*4 h*	
		Control	*2 mmol acetic acid metabolite/ litre*	*Control*	*2 mmol acetic acid metabolite/ litre*	*Control*	*2 mmol acetic acid metabolite/ litre*
Younger adults	9	0.5 ± 0.2	0.3 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	1.0 ± 0.5	0.7 ± 0.2
Older adults	9	0.7 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	1.3 ± 0.4	1.2 ± 0.2	1.4 ± 0.4
Sickle cell disease	7	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.4 ± 0.4	1.2 ± 0.2
Spherocytosis	3	1.0 ± 0.5	0.9 ± 0.4	1.3 ± 0.5	1.4 ± 0.3	2.3 ± 0.6	2.3 ± 0.4

[a] Mean ± standard error of the mean (SEM) from the number of samples available. Control medium was buffer containing 10 mmol Tris/litre (pH 7.4), 140 mmol sodium chloride/litre, 2 mmol calcium chloride/litre, 4 mmol potassium chloride/litre and 0.1% bovine albumin. Incubations were performed at 37 °C in a shaking water bath.

There was some degree of haemolysis in the presence of buffer alone with the cells from older people, and those with genetic disorders were slightly more prone to haemolysis. However, in all cases, incubation with acetic acid metabolite at 2 mmol/litre had no effect on the amount of haemolysis observed.

Development of a CSAF for interspecies differences in toxicokinetics (AK_{AF})

Identification of the active chemical moiety

Observed variations in toxicity with sex, age, duration and species correlated well with differences in production and clearance of the acetic acid metabolite. These observations and additional studies in which oxidation of Compound B to the corresponding acid was inhibited indicate that the acid metabolite is principally responsible for the haematological effects observed in experimental animals exposed to the compound. Toxicokinetic data were for the acetic acid metabolite.

Choice of relevant toxicokinetic parameter

In the absence of information to allow meaningful selection of the appropriate toxicokinetic parameter, the more conservative AUC is considered appropriate, although the C_{max} could be more relevant for a direct effect. Since the data relate to single exposure, the AUC in humans was extrapolated to infinity.

Experimental data in animals

1) *Relevance of route:*

   The AUCs were calculated from inhalation data and are, therefore, appropriate for the relevant route of exposure in humans.

2) *Relevance of dose/concentration:*

   The lowest dose at which plasma toxicokinetic data were obtained in rats was slightly higher than the BMCs for haematological effects in this species. If saturation of metabolism were to occur between the BMC and the dose used in the kinetic studies, it would tend to increase the AUC of the parent compound and therefore reduce the difference between rats and humans. There was a linear relationship between the concentration inhaled and the AUC at the doses studied.

3) *Adequacy of number of subjects/samples:*

   The data were for 16 animals at each time point, and the calculated AUC and clearance were based on the mean values and represented a good estimate of the central tendency.

Experimental data in humans

1) *Relevance of population:*

   The group studied comprised healthy adult males. It is noted that females were not included in experimental studies; although female animals were slightly more sensitive than males, the data for males were considered acceptable.

2) *Relevance of route:*

   The AUCs were calculated from inhalation data and are, therefore, appropriate for the relevant route of exposure.

3) *Relevance of concentration:*

   The dose administered was 97 mg/m^3, which is similar to or greater than the BMCs for haematological effects in animals; it is 20-fold greater than the critical BMC in animal studies.

4) *Adequacy of number of subjects/samples:*

   The plasma toxicokinetic data in humans were for five individuals for which little information on variability was presented. While available data did not permit calculation of the standard error, the number of individuals meets the minimum number recommended and appears to be sufficient in view of the seemingly low variability in the data.

Calculation of a CSAF for interspecies differences in toxicokinetics (AK_{AF})

Use of output of the PBPK models as an appropriate basis for development of an AK_{AF} was considered justified, primarily on the basis of their mathematical validation. However, due to the non-validated biological assumptions inherent in the models, particularly in relation to the renal handling of the key acetic acid metabolite, they add little additional value to scaling of basic kinetic parameters, such as AUC or clearance, by (body weight)$^{0.7}$. Hence, a simple comparison of the AUCs for humans and experimental animals for the active metabolite is as informative for interspecies scaling.

Interspecies scaling is based, in part, on relevant data from the kinetic study in five human volunteers described above. The tabulated mean data were analysed using a non-compartmental model and a standard kinetic program (WinNonLin), which gave an AUC extrapolated to infinity of 414 (μmol/litre)·h. Since these relate to a 2-h exposure to 97 mg/m^3, the AUC is 414/(2 × 97) = 2.13 (μmol/litre)·h / (mg/m^3)·h. Studies performed with the PBPK model indicated that the uptake of the parent compound in this experiment would be linearly related to the ventilation rate. Therefore, adjustment of the AUC value to account for working versus resting conditions (for comparison with the AUC in rats) (15 m^3/day / 57 m^3/day to account for ventilation rate at 50 W work versus resting conditions) gives an AUC of 0.56 (μmol/litre)·h / (mg/m^3)·h. Note that the need to readjust the uptake of the highly soluble

Compound B for the ventilation rate contrasts with the case of the poorly soluble Compound C (see below), where ventilation rate has little impact on uptake.

AUCs in the critical animal study in which groups of male and female rats were exposed to three concentrations for up to 18 months were reported for the post-exposure period only. Therefore, the AUC in animals for the exposure and post-exposure period in this study was calculated on the basis of the PBPK model described above. The concentration of acetic acid metabolite in venous blood at 303 mg/m^3 for an inhalation time of 6 h in each 24 h was 2077.5 [(μmol/litre)·h] / (303 mg/m^3 · 6 h) = 1.14 [(μmol/litre)·h] / (mg/m^3)·h.

The resulting ratio between the AUC for humans and that for rats is 0.56/1.14 or 0.49, which is approximately one-eighth of the default value for this component (i.e., 4.0).

Development of a CSAF for interspecies differences in toxicodynamics (AD_{AF})

Identification of the active chemical moiety

Observed variations in toxicity with sex, age, duration and species correlated well with differences in production and clearance of the acetic acid metabolite. These observations and additional studies in which oxidation of Compound B to the corresponding acid was inhibited indicate that the acid metabolite is principally responsible for the haematological effects observed in experimental animals exposed to the compound. Toxicodynamic data in humans and animals were for the acetic acid metabolite.

Consideration of end-point

Equipotent concentrations could not be calculated from the available *in vitro* data, but these were sufficient to show that there was at least one order of magnitude difference in sensitivity.

Experimental data in animals

1) *Relevance of population; adequacy of number of subjects/samples:*

   In the relevant study reported in most detail above, data are presented for haemolysis in three male and three female animals at each dose level. In addition, there are data from four other animals in another study and an unspecified number of animals in a further investigation. Standard errors (i.e., standard deviations divided by the square root of the sample numbers) are less than 20% of the mean.

2) *Adequacy of concentration–response:*

   Erythrocytes from rats in the same study were exposed to three dose levels, in addition to controls.

Experimental data in humans

1) *Relevance of population; adequacy of number of subjects/samples:*

   The data relevant to assessment of interspecies differences in toxicodynamics in the study reported in most detail above are for five men and women, 18–40 years of age. In addition, there are data from pooled human erythrocytes from two other studies, although little information on the origin of these samples was provided. Standard errors (i.e., standard deviations divided by the square root of the sample numbers) in the study reported in most detail are less than 20% of the mean.

2) *Adequacy of concentration–response:*

   There were data on three dose levels and controls in humans in the study presented in most detail above.

Calculation of a CSAF for interspecies differences in toxicodynamics (AD_{AF})

Although it is not possible to derive an EC_{10} value from the available data for the effect of the acetic acid metabolite on human blood, the data indicate clear effects at 0.5 mmol/litre with rat blood and negligible effects at 8.0 mmol/litre with human blood. Therefore, there is good evidence that human erythrocytes are at least 10-fold less sensitive than rat erythrocytes; therefore, the default factor for the interspecies component for dynamics (2.5) can be replaced with a value of 0.1 (and this would still be conservative).

Development of a CSAF for human variability in toxicokinetics (HK_{AF})

Identification of the active chemical moiety

Observed variations in toxicity with sex, age, duration and species correlated well with differences in production and clearance of the acetic acid metabolite. These observations and additional studies in which oxidation of Compound B to the corresponding acid was inhibited indicate that the acetic acid metabolite is principally responsible for the haematological effects observed in experimental animals exposed to the compound. Toxicokinetic data in humans were for the acetic acid metabolite.

Choice of relevant toxicokinetic parameter

The more conservative AUC is selected as the appropriate toxicokinetic parameter, for which the single-dose kinetics data in humans can be extrapolated to infinity.

Experimental data

Although the data on toxicokinetics in humans (restricted to five male volunteers) are sufficient as a basis for determination of a measure of central tendency for interspecies comparison, they are considered inadequate to define the potential variability in the human

population.[1] An analysis of the population distribution of clearance in a relatively large population (depending on the incidence of any polymorphism) would be necessary.

Calculation of a CSAF for human variability in toxicokinetics (HK_{AF})

An analysis of the population distribution would be necessary as a basis for the CSAF for human variability in toxicokinetics (HK_{AF}). The number of subjects for which there are data is inadequate as a basis for characterization of the population distribution and, hence, is inadequate as a basis for development of an HK_{AF}. Thus, the default uncertainty factor of 3.16 is maintained.

Development of a CSAF for human variability in toxicodynamics (HD_{AF})

Identification of the active chemical moiety

Observed variations in toxicity with sex, age, duration and species correlated well with differences in production and clearance of the acetic acid metabolite. These observations and additional studies in which oxidation of Compound B to the corresponding acid was inhibited indicate that the acetic acid metabolite is principally responsible for the haematological effects observed in experimental animals exposed to the compound. Toxicodynamic data relevant to variability in humans were for the acetic acid metabolite.

Consideration of end-point

The data were not amenable to the calculation of an EC_{10} or any other value suitable to define the concentration–response relationship.

Experimental data

1) *Relevance of population; adequacy of number of subjects/samples:*

   In the study in which haemolysis in potentially susceptible subgroups of the population was investigated, cells were obtained from 18 healthy individuals, 9 with a mean age of 41 years (range 31–56 years; 5 men, 4 women) and 9 with a mean age of 72 years (range 64–79 years; 5 men, 4 women), from 7 people with sickle cell disease and from 3 people with spherocytosis.

[1] For example, there is a well recognized genetic polymorphism for alcohol dehydrogenases (the group of enzymes responsible for the first step in the metabolism of Compound B to the acetic acid metabolite); however, while this is relevant to replacement of the default for interindividual variation, the proportion of the population affected is small and would not meaningfully impact on the measure of central tendency; in addition, the specific isozyme involved in metabolizing Compound B is not known.

2) *Adequacy of concentration–response:*

Data were restricted to a single dose level and controls in the study in which haemolysis in erythrocytes of potentially susceptible human population subgroups was examined, and variability in EC_{10} values could not be determined.

Calculation of a CSAF for human variability in toxicodynamics (AD_{AF})

Available data on human variability in dynamics are limited primarily to one study *in vitro* in blood from various potentially susceptible subgroups of the population in which no response was observed at the administered concentration (n = 9, 9, 7 and 3). In several other studies, haemolysis was examined in pooled blood samples from unspecified or small numbers of individuals (n = 5) as a basis solely for estimation of the central tendency for interspecies differences. These data are inadequate to quantitatively inform the replacement of default with a CSAF, and, hence, the default value of 3.16 is maintained.

Calculation of the composite uncertainty factor (CUF)

The CUF is a composite of the adjustment factors for interspecies differences in toxicokinetics and toxicodynamics and the default uncertainty factors for human variability in toxicokinetics and toxicodynamics. The CUF is generally shown as:

$$CUF = [AK_{AF} \text{ or } AK_{UF}] \times [AD_{AF} \text{ or } AD_{UF}] \times [HK_{AF} \text{ or } HK_{UF}] \times [HD_{AF} \text{ or } HD_{UF}]$$

In this case:

$$CUF = AK_{AF} \times AD_{AF} \times HK_{UF} \times HD_{UF}$$

The CUF is, therefore, $0.49 \times 0.1 \times 3.16 \times 3.16 = 0.5$.

Because of this low CUF, other effects detected in animal studies at higher inhaled concentrations should be considered, because these are likely to become the critical effect for defining a health-based risk estimate.

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CASE C: Development of HK_{AF}

Preamble

Exposure of humans to Compound C is from inhalation only.

Observed variations in liver toxicity in humans and laboratory animals correlated well with differences in production and clearance of the acid metabolite of Compound C. These data, supported by studies in which oxidation of the parent compound to the corresponding acid was inhibited, indicate that the acid metabolite is most likely the active moiety.

Data collected during and following exposure of laboratory animals and humans to the parent chemical demonstrate that it is readily absorbed from air into blood and that it is rapidly cleared from blood following the cessation of exposure.

Metabolism proceeds via the hepatic P450 enzyme system to produce a short-lived aldehyde intermediate. Results from *in vivo* studies in humans indicate that about 10% of the aldehyde intermediate is then further reduced by aldehyde reductase to an alcohol metabolite, which is then conjugated with uridine diphosphoglucuronic acid via an unidentified form of uridine diphosphate glucuronyl transferase prior to its elimination in urine. Approximately 90% of the aldehyde intermediate is oxidized by aldehyde dehydrogenase to form the putative hepatotoxic acid metabolite. *In vitro* data also indicate that approximately 90% of the aldehyde metabolite is rapidly converted to the acid metabolite.

The rapidity with which the aldehyde intermediate is further metabolized is evidenced by the fact that humans exposed to 655 mg/m^3 of the parent chemical demonstrated appreciable levels of the acid metabolite and the alcohol metabolite in blood, but the intermediate aldehyde metabolite was not detected.[1] Following oral exposure of the rat, mouse and human to the intermediate aldehyde metabolite, it was subject to extensive first-pass metabolism and exhibited a short plasma half-life. Following the intravenous administration of the aldehyde intermediate to mice at doses of approximately 10 mg/kg body weight, no aldehyde was detectable in blood within 10 min. Further metabolic conversion of the acid metabolite is minimal.

Human variability in toxicokinetics

The focus of this case-study is the interpretation and use of data on variability in metabolism by human tissues *in vitro*.

Adult males and females were exposed by inhalation to 546 mg/m^3 of the parent chemical for 4 h, and data on concentrations of the parent chemical and metabolites in blood, urine and breath were determined for up to 2 days post-exposure. Concentrations of the parent chemical in alveolar breath were determined immediately following the exposure, and concentrations of parent chemical and acid metabolite were determined in blood and urine during the exposure period and to 2 days post-exposure. Table A-11 summarizes post-exposure concentrations of the parent chemical in blood and in the exhaled breath immediately post-exposure. In Tables A-12 and A-13, data on levels of the acid metabolite in blood and urine are presented.

Because uptake of the chemical into blood was governed by the biochemistry determining partitioning of the parent chemical from air into blood, individual blood:air partition coefficients were derived (Table A-14). These data indicate that there was minimal interindividual variability in partitioning of the chemical into blood. The mean ± SD of the blood:air partition coefficient for seven adult males and six adult females was 11.15 ± 0.74 and 9.13 ± 1.73, respectively. For this poorly water soluble compound, ventilation has little impact on

[1] Conversion factor in air for Compound C: 1 ppm = 5.46 mg/m^3.

uptake, because steady state is achieved quite rapidly, after which ventilatory uptake is determined by metabolic clearance. The similarity of partition coefficients across individuals suggests that humans exposed to the same concentrations of the parent chemical will have similar blood concentrations.

Table A-11. Post-exposure concentrations of parent chemical in blood and exhaled breath.

Subject	*Post-exposure concentration of parent chemical in blood (µg/ml)*			*Exhaled breath immediately post-exposure (mg/m^3)*		
	Female (n = 10)	*Male (n = 11)*	*Combined (n = 21)*	*Female (n = 8)*	*Male (n = 9)*	*Combined (n = 17)*
1	2.3	2.1		11	66	
2	1.6	4.5		60	82	
3	1.45	2.6		76	87	
4	2.7	2.8		87	98	
5	3.0	4.25		98	104	
6	1.2	3.75		104	109	
7	1.1	2.25		115	115	
8	1.2	2.8		120	115	
9	1.6	4.55			137	
10	2.05	2.65				
11		1.8				
Mean	1.82	3.1	2.49	84.1	102	93.4
SD	0.66	0.99	1.06	35.5	213	28.9

Table A-12. Acid metabolite concentrations in human blood and urine.

	Peak concentration in blood (µg/ml)	*Time to attain peak concentration in blood[a] (min)*		*Cumulative acid metabolite in urine to 2 days (mg)[b]*	
		Mean ± SD	*Range*	*Mean ± SD*	*Range*
Females	9.7 ± 1.9	34 ±13	20–51	71 ± 23	46–112
Males	9.2 ± 1.6	44 ±15	22–47	68 ± 21	46–102

[a] Near-maximum concentrations were maintained for 1–2 days.
[b] Individual data presented below in Table A-13.

The parent chemical is metabolized to the aldehyde intermediate by CYP2E1, and this is the rate-limiting step in the formation of the active acid metabolite. Immunoquantification of CYP2E1 in microsomal samples (obtained from 140 organ donors) was accomplished by enzyme-linked immunosorbent assay (ELISA) (see Table A-15). Forty per cent of the donors were female and 60% male; 80% were Caucasian, 9% were Hispanic, 4% were Black and 1%

were Asian. The mean age was 47, with a standard deviation of 14 years; the youngest donor was 6 years of age and the oldest 70 years.

Table A-13. Forty-eight-hour cumulative urinary elimination of acid metabolite in human volunteers.

Subject	*Acid metabolite eliminated in urine (mg)*	
	Female	*Male*
1	51.9	80.6
2	71.1	78.7
3	55.9	45.6
4	54.1	51.6
5	51.7	48.7
6	45.7	54.9
7	91.9	102.0
8	82.3	67.0
9	111.8	86.8
10	97.5	60.7
11		93.2
Mean ± SD	71 ± 23	68 ± 21

Table A-14. Blood:air partition coefficient values for individual human volunteers.

Subject	*Blood:air partition coefficient*	
	Female	*Male*
1	9.88	11.49
2	9.45	10.10
3	6.47	11.10
4	7.63	12.10
5	11.00	11.91
6	10.37	10.85
7		10.47
Mean ± SD	9.13 ± 1.73	11.15 ± 0.74

For 23 of these samples, the Michaelis-Menten kinetic constants (K_m), which govern the metabolism of the parent chemical to the aldehyde metabolite, were also measured. The metabolic rate (theoretical maximal initial rate of metabolism, V_{max}) for the CYP2E1-dependent metabolism of the parent chemical was determined through carefully controlled *in vitro* exposures of the microsomal protein and determination of the rate of formation of the aldehyde intermediate. Because the exact content of CYP2E1 in these microsomal samples was known from the results of an experiment summarized in Table A-15, the V_{max} value has

been expressed per unit of enzyme (pmol/min per pmol CYP2E1), in addition to the less specific measure of "per mg microsomal protein" (see Table A-16).

Table A-15. Distribution of CYP2E1 protein in 140 human hepatic microsomal samples.

Parameter	*CYP2E1 protein*[a]
Mean	59.36
Standard error	1.535
Median	57.5
Mode	58
Standard deviation	18.16
Sample variance	330
Range	107
Minimum	23
Maximum	130
Count	140

[a] Enzyme content (pmol CYP2E1/mg microsomal protein) was determined by ELISA in microsomal samples prepared from the livers of 140 human organ donors. The lower 5% confidence limit (5% LCL) for these data was 25 pmol CYP2E1/mg microsomal protein, and the upper 95% confidence limit (95% UCL) for these data was 125 pmol CYP2E1/mg microsomal protein.

A study was also conducted to determine the yield of microsomal protein from intact liver derived from adult organ donors. The study involved four human livers and assessed the activity of glucose-6-phosphatase in crude liver homogenate and in microsomes prepared from the same livers. The results indicated that microsomal protein (which contains the CYP enzymes) was present in these samples at a mean value of 20.8 (± 5.0 SD) mg microsomal protein per gram intact liver. These data were used in the construction of a PBPK model for humans (see section 3).

A six-compartment PBPK model was constructed for the parent chemical, with compartments assigned to lung, liver, kidney, fat, rapidly perfused tissues and slowly perfused tissues. Because of the high degree of lipophilicity of the parent chemical, the volume of the fat compartment for the model was determined by measuring the percentage of body fat in each of the eight female and nine male participants. This volume was 10–27% for males and 21–35% for females. Tissue volumes were obtained from published sources. Tissue blood flows, expressed as a percentage of the cardiac output, were obtained from the literature and used as single (point) values for both males and females.

Tissue:air partition coefficients for the parent chemical were obtained for liver, kidney, skeletal muscle, lung and fat through the assessment of an unspecified number of female organ donors, with only one tissue type assessed per individual organ donor. Neither the number of samples assessed nor data on the variability of tissue:air partition coefficients were presented. In addition to these partitioning data, blood was drawn from the actual study

participants and used to determine the blood:air partition coefficient for the parent chemical (see Table A-14 above). Partitioning of the parent chemical from blood into tissues was determined by dividing the mean (by sex) blood:air partition coefficient by the tissue:air partition coefficient derived for each tissue type. Alveolar ventilation rate and partitioning of parent chemical from air into blood were used to determine the concentration of chemical entering the bloodstream, assuming equilibration of the chemical between the blood and alveolar air. Distribution from blood to tissues was assumed to be blood flow-limited. The liver was assumed to be the site of metabolism, which was described using Michaelis-Menten kinetics.

Table A-16. Derivation of the specific activity of CYP2E1 towards the parent chemical.

Sample	*pmol CYP2E1/mg microsomal protein*	*Specific activity*[a]	*V_{max} for metabolism of parent chemical*[b]
1	33	21.0	694
2	32	29.8	955
3	39	28.9	1128
4	52	24.6	1280
5	59	23.2	1367
6	88	16.8	1477
7	22	27.5	606
8	84	23.8	1996
9	92	15.1	1389
10	64	22.4	1432
11	66	12.8	846
12	39	35.1	1367
13	63	21.4	1347
14	36	33.2	1194
15	55	15.4	846
16	39	12.2	477
17	44	24.6	1083
18	96	23.7	2279
19	105	18.2	1910
20	88	11.6	1020
21	47	33.7	1584
22	73	26.2	1910
23	88	10.2	899
Mean		22.2	1265
SD		7.3	462

[a] Data on specific activity are presented as pmol parent chemical metabolized per minute per pmol CYP2E1.

[b] Data on V_{max} are presented as pmol parent chemical metabolized per minute per mg microsomal protein. CYP2E1 is one component of microsomal protein.

PBPK models were developed and fitted to human data that were collected during and following a 4-h inhalation exposure to the chemical (presented in Tables A-11 to A-13).

The extrapolation of the *in vitro*-derived metabolic rates to units appropriate for inclusion in PBPK models relied on several factors: 1) the specific activity towards the parent chemical (nmol/min per mg microsomal protein), 2) the quantity of microsomal protein present per gram intact liver and 3) the fraction of body weight attributable to liver.

In carefully conducted studies with samples of human liver, the mean specific activity (apparent V_{max} value) for CYP2E1 protein towards the parent chemical was 22 ± 7 (5% LCL = 8; 95% UCL = 38) pmol parent chemical/min per pmol CYP2E1 (see Table A-16); liver microsomal protein contained 59 ± 18 (5% LCL = 25; 95% UCL = 125) pmol CYP2E1/mg microsomal protein (see Table A-15). As discussed above, intact liver contains 21 ± 5 mg (mean ± SD, n = 4) microsomal protein per gram. For extrapolation purposes (below) and to ensure consistency with the values incorporated as parameters in the PBPK model, liver mass was formalized to 2.6% body mass.

These metabolic rates were then converted (by molecular weight and time conversions) into units in which metabolic rate is expressed in PBPK models (mg/h per kg body weight), as shown below:

- *In vivo* metabolic rate extrapolated from the 5th percentile for the *in vitro* V_{max}:

5th percentile for *in vitro* V_{max} value:	Mean − (2SD) 1265 − (2 × 462) = 341 pmol/min per mg microsomal protein
	341 pmol parent chemical metabolized/min per mg microsomal protein
	× 131.5 ng/nmol (molecular weight)
	÷ 1 000 000 ng/mg
	÷ 1000 pmol/nmol
	× 21 mg microsomal protein/g liver
	× 60 min/h
	× 26 g liver/kg body weight
	= 1.47 mg/h per kg body weight

- Metabolic rate calculated at the mean value for enzyme content and specific activity:

Mean *in vitro* V_{max} value:	1265 pmol/min per mg microsomal protein
	× 131.5 ng/nmol (molecular weight)
	÷ 1 000 000 ng/mg
	÷ 1000 pmol/nmol
	× 21 mg microsomal protein/g liver
	× 60 min/h
	× 26 g liver/kg body weight

= 5.45 mg/h per kg body weight

In vivo metabolic rate extrapolated from the 95th percentile for *in vitro* V_{max}:

95th percentile for *in vitro* V_{max} value:	Mean + (2SD) 1265 + (2 × 462) = 2189 pmol/min per mg microsomal protein
	2189 pmol parent chemical metabolized/min per mg microsomal protein × 131.5 ng/nmol (molecular weight) ÷ 1 000 000 ng/mg ÷ 1000 pmol/nmol × 21 mg microsomal protein/g liver × 60 min/h × 26 g liver/kg body weight = 9.43 mg/h per kg body weight

Tables A-17 and A-18 demonstrate that despite the variability in intrinsic liver clearances with respect to the production of the hepatotoxic acid metabolite, there is little difference throughout the population in the amount of compound metabolized following inhalation.

Table A-17. Impact of calculated human variance of CYP2E1 expression (activity) on the amount of chemical metabolized.

Enzyme activity	*Amount metabolized (mg/litre)[a] at following exposure concentrations:*		
	2.7 mg/m³	*27 mg/m³*	*270 mg/m³*
5th percentile (1.47 mg/h per kg body weight)	1.16	11.1	52.8
95th percentile (9.43 mg/h per kg body weight)	1.39	13.9	136.5
Magnitude of difference	1.20	1.25	2.59

[a] Amount of chemical metabolized per mass (litre) of liver.

The apparent discrepancy between the large variation in metabolic capacity and the small increase in metabolite formation is a consequence principally of the rate of delivery of the parent chemical to the liver relative to the high intrinsic metabolic capacity of the liver for the chemical; metabolism was flow-limited at exposures below maximal occupational exposure conditions (i.e., in and above the range predicted for environmental and residential exposures). Increases in alveolar ventilation rate had minimal impact, and this was attributed to the poor solubility of the chemical in human blood. Increases in hepatic blood flow had more impact than increases in alveolar ventilation rate, but physiological limitations to the magnitude of these limited the increase in metabolite formation to a maximum of 2.6-fold in the face of a 6.4-fold increase in metabolic capacity. Under less than maximally permitted

occupational exposures, this increase in metabolic capacity resulted in an approximate 25% increase in metabolite formation.

Table A-18. Impact of human variability of CYP2E1 expression (activity) on the concentration of the acid metabolite in blood 1 h post-exposure.

Enzyme activity	*Concentration of metabolite (µg/ml)[a] at following exposure concentrations:*		
	2.7 mg/m³	*27 mg/m³*	*270 mg/m³*
5th percentile (1.47 mg/h per kg body weight)	0.007	0.07	0.29
95th percentile (9.43 mg/h per kg body weight)	0.008	0.08	0.61
Magnitude of difference	1.14	1.14	2.10

[a] Concentration of acid metabolite measured; data expressed as µg/ml of whole blood.

Development of a CSAF for human variability in toxicokinetics (HK_{AF})

Identification of the active chemical moiety

As stated in the preamble, observed variations in liver toxicity in humans and laboratory animals correlated well with differences in production and clearance of the acid metabolite. These data, supported by studies in which oxidation of the parent compound to the corresponding acid was inhibited, indicate that the acid metabolite is the active chemical moiety. This metabolite is formed in the liver via CYP2E1 from the intermediate aldehyde metabolite. About 90% of the aldehyde is readily oxidized to the acid metabolite, which does not undergo any further metabolism. The acid metabolite has been detected in the blood and urine of exposed animals and humans. Although the aldehyde is also reduced to an alcohol, which undergoes further conjugation followed by urinary excretion, this is a minor pathway (10%), and these pathways do not appear to contribute to the hepatic toxicity of the compound.

Choice of relevant toxicokinetic parameter

Use of the AUC of either the parent chemical or the acid metabolite as the kinetic parameter is considered most appropriate. However, given that the elimination half-lives for the parent chemical and acid metabolite are relatively rapid (hours), the peak blood concentrations for either the parent chemical or the active acid metabolite are also considered acceptable as surrogate toxicokinetic parameters for determining interindividual differences in toxicokinetics. Total measures of urinary excretion of the acid metabolite are considered inappropriate, because although they reflect the total amount of the acid metabolite formed, they do not reflect the concentrations available at the target organ (liver).

Experimental data

1) *Relevance of population:*

The group of humans studied was composed of healthy adult males and females and therefore was of relevance to the effect of interest (i.e., liver toxicity from the acid metabolite).

2) *Relevance of route:*

The adults from whom relevant data (Tables A-11 and A-12) were obtained were exposed via inhalation. The inhalation and the oral routes have been utilized in animal studies demonstrating liver toxicity and its dependence upon metabolism, and the inhalation route is anticipated to be the primary exposure route for humans. Because of the existence of well documented PBPK models for humans and animals for this chemical, internal concentrations from oral or inhalation routes of exposure could have been attained; it is these concentrations that determine metabolic activation.

3) *Relevance of dose:*

Humans expressing the relevant enzyme at only low levels are still able to metabolize this chemical efficiently, given the limiting rates at which it is delivered to the liver. A greater magnitude of interindividual difference in metabolite formation (and toxicity) would become evident only under acutely toxic doses, such that metabolism was saturated. Below these doses, the toxicokinetics of this agent are independent of dose; the doses employed in the human studies produced data relevant to similar (occupationally relevant) and lower (environmentally relevant) exposures.

4) *Adequacy of number of subjects/samples:*

In contrast to measures of central tendency, the goal of this study was to estimate variance. Data in Tables A-11 and A-12 (peak blood level of the parent chemical and peak blood level of the acid metabolite, respectively) are from 11 male and 10 female healthy adult volunteers. The standard error (standard deviation [SD] of the sample divided by the square root of the sample size) of measures of the parent chemical in blood (Table A-11; $1.06/21^{0.5} = 0.23$) is only 9% of the mean (2.49) and is therefore acceptable as a measure of the central tendency. Further, the PBPK model employed measures of the variance of the enzyme responsible for metabolic activation, as determined from 140 human organ donors. The incorporation of these extrapolated bounds of variance (i.e., 5th and 95th percentiles; see Tables A-17 and A-18) does not result in any increase in the extent of interindividual variation in the formation of the toxic acid metabolite. These data are considered adequate to account for interindividual differences in toxicokinetics in the general human population. As discussed below, the PBPK model could be utilized to address additional variability among the general population and susceptible population subgroups.

Calculation of a CSAF for human variability in toxicokinetics (HK_{AF})

Based on the data in Table A-11 for concentrations in exhaled breath, the HK_{AF} is 1.6 (mean plus two standard deviations divided by mean = [17.1 + 2(5.3)] / 17.1 = 27.7/17.1 = 1.6). Gender differences are not apparent. Thus, the data for males and females could be combined.

Susceptible population subgroups

The HK_{AF} value derived above is applicable for the general population. Additional data are required to address variability in any susceptible population subgroup.

Variation across the human population is likely best reflected by variation in blood flow (to the liver) and renal clearance. Even though the *in vitro* data indicate that there is a wide variation in capacity of human livers to metabolize the parent chemical, the PBPK model indicates that the *in vivo* metabolism of the parent chemical is limited by the liver blood flow. That is, the rate of blood flow determines how much of the parent chemical is delivered to the liver. Blood flow may vary with age and health status, and the HK_{AF} can be refined based on information on variation in blood flow in susceptible subgroups.

In addition, renal clearance may vary with age and health status, as the active acid metabolite is eliminated primarily in the urine. Impairment of renal function may result in more pronounced liver toxicity of this chemical, as the elimination of the active metabolite will be reduced.

In the absence of *in vivo* data, the PBPK model would be useful in predicting human variation in toxicokinetics.

APPENDIX 2: GLOSSARY OF TERMS

Alphabetical list of selected key generic terms in hazard and risk assessment and their definitions.[1]

Term	*Description*
Acceptable daily intake	Estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub)population may be exposed daily over their lifetimes without appreciable health risk. Related terms: *Reference dose, Tolerable daily intake*
Acceptable risk	This is a risk management term. The acceptability of the risk depends on scientific data, social, economic, and political factors, and the perceived benefits arising from exposure to an agent.
Adverse effect	Change in the morphology, physiology, growth, development, reproduction, or life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.
Analysis	Detailed examination of anything complex, made in order to understand its nature or to determine its essential features.
Assessment	Evaluation or appraisal of an analysis of facts and the inference of possible consequences concerning a particular object or process.
Assessment end-point	Quantitative/qualitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment.
Assessment factor	Numerical adjustment used to extrapolate from experimentally determined (dose–response) relationships to estimate the agent exposure below which an adverse effect is not likely to occur. Related terms: *Safety factor, Uncertainty factor*
Concentration	Amount of a material or agent dissolved or contained in unit quantity in a given medium or system.
Concentration–effect relationship	Relationship between the exposure, expressed in concentration, of a given organism, system, or (sub)population to an agent in a specific pattern during a given time and the magnitude of a continuously graded effect to that organism, system, or (sub)population. Related terms: *Effect assessment, Dose–response relationship*

[1] Table taken from IPCS (2004).

Term	*Description*
Dose	Total amount of an agent administered to, taken up by, or absorbed by an organism, system, or (sub)population.
Dose–effect relationship	Relationship between the total amount of an agent administered to, taken up by, or absorbed by an organism, system, or (sub)population and the magnitude of a continuously graded effect to that organism, system, or (sub)population. Related terms: *Effect assessment, Dose–response relationship, Concentration–effect relationship*
Dose-related effect	Any effect to an organism, system, or (sub)population as a result of the quantity of an agent administered to, taken up by, or absorbed by that organism, system, or (sub)population.
Dose–response	Relationship between the amount of an agent administered to, taken up by, or absorbed by an organism, system, or (sub)population and the change developed in that organism, system, or (sub)population in reaction to the agent. Synonymous with *Dose–response relationship*. Related terms: *Dose–effect relationship, Effect assessment, Concentration–effect relationship*
Dose–response assessment	Analysis of the relationship between the total amount of an agent administered to, taken up by, or absorbed by an organism, system, or (sub)population and the changes developed in that organism, system, or (sub)population in reaction to that agent, and inferences derived from such an analysis with respect to the entire population. Dose–response assessment is the second of four steps in risk assessment. Related terms: *Hazard characterization, Dose–effect relationship, Effect assessment, Dose–response relationship, Concentration–effect relationship*
Dose–response curve	Graphical presentation of a dose–response relationship.
Dose–response relationship	Relationship between the amount of an agent administered to, taken up by, or absorbed by an organism, system, or (sub)population and the change developed in that organism, system, or (sub)population in reaction to the agent. Related terms: *Dose–effect relationship, Effect assessment, Concentration–effect relationship*
Effect	Change in the state or dynamics of an organism, system, or (sub)population caused by the exposure to an agent.

Term	*Description*
Effect assessment	Combination of analysis and inference of possible consequences of the exposure to a particular agent based on knowledge of the dose–effect relationship associated with that agent in a specific target organism, system, or (sub)population.
Expert judgement	Opinion of an authoritative person on a particular subject.
Exposure	Concentration or amount of a particular agent that reaches a target organism, system, or (sub)population in a specific frequency for a defined duration.
Exposure assessment	Evaluation of the exposure of an organism, system, or (sub)population to an agent (and its derivatives). Exposure assessment is the third step in the process of risk assessment.
Exposure scenario	A set of conditions or assumptions about sources, exposure pathways, amounts or concentrations of agent(s)involved, and exposed organism, system, or (sub)population (i.e., numbers, characteristics, habits) used to aid in the evaluation and quantification of exposure(s) in a given situation.
Fate	Pattern of distribution of an agent, its derivatives, or metabolites in an organism, system, compartment, or (sub)population of concern as a result of transport, partitioning, transformation, or degradation.
Guidance value	Value, such as concentration in air or water, that is derived after allocation of the reference dose among the different possible media (routes) of exposure. The aim of the guidance value is to provide quantitative information from risk assessment to the risk managers to enable them to make decisions. (See also *Reference dose*)
Hazard	Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system, or (sub)population is exposed to that agent.
Hazard assessment	A process designed to determine the possible adverse effects of an agent or situation to which an organism, system, or (sub)population could be exposed. The process includes hazard identification and hazard characterization. The process focuses on the hazard, in contrast to risk assessment, where exposure assessment is a distinct additional step.

Term	*Description*
Hazard characterization	The qualitative and, wherever possible, quantitative description of the inherent property of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose–response assessment and its attendant uncertainties. Hazard characterization is the second stage in the process of hazard assessment and the second of four steps in risk assessment. Related terms: *Dose–effect relationship, Effect assessment, Dose–response relationship, Concentration–effect relationship*
Hazard identification	The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system, or (sub)population. Hazard identification is the first stage in hazard assessment and the first of four steps in risk assessment.
Margin of exposure	Ratio of the no-observed-adverse-effect level (NOAEL) for the critical effect to the theoretical, predicted, or estimated exposure dose or concentration. Related term: *Margin of safety*
Margin of safety	For some experts, margin of safety has the same meaning as margin of exposure, while for others, margin of safety means the margin between the reference dose and the actual exposure. Related term: *Margin of exposure*
Measurement end-point	Measurable (ecological) characteristic that is related to the valued characteristic chosen as an assessment point.
Reference dose	An estimate of the daily exposure dose that is likely to be without deleterious effect even if continued exposure occurs over a lifetime. Related term: *Acceptable daily intake*
Response	Change developed in the state or dynamics of an organism, system, or (sub)population in reaction to exposure to an agent.
Risk	The probability of an adverse effect in an organism, system, or (sub)population caused under specified circumstances by exposure to an agent.
Risk analysis	A process for controlling situations where an organism, system, or (sub)population could be exposed to a hazard. The risk analysis process consists of three components: risk assessment, risk management, and risk communication.

Term	*Description*
Risk assessment	A process intended to calculate or estimate the risk to a given target organism, system, or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The risk assessment process includes four steps: hazard identification, hazard characterization (related term: *Dose–response assessment*), exposure assessment, and risk characterization. It is the first component in a risk analysis process.
Risk characterization	The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system, or (sub)population, under defined exposure conditions. Risk characterization is the fourth step in the risk assessment process.
Risk communication	Interactive exchange of information about (health or environmental) risks among risk assessors, managers, news media, interested groups, and the general public.
Risk estimation	Quantification of the probability, including attendant uncertainties, that specific adverse effects will occur in an organism, system, or (sub)population due to actual or predicted exposure.
Risk evaluation	Establishment of a qualitative or quantitative relationship between risks and benefits of exposure to an agent, involving the complex process of determining the significance of the identified hazards and estimated risks to the system concerned or affected by the exposure, as well as the significance of the benefits brought about by the agent. Risk evaluation is an element of risk management. Risk evaluation is synonymous with risk–benefit evaluation.
Risk management	Decision-making process involving considerations of political, social, economic, and technical factors with relevant risk assessment information relating to a hazard so as to develop, analyse, and compare regulatory and non-regulatory options and to select and implement appropriate regulatory response to that hazard. Risk management comprises three elements: risk evaluation; emission and exposure control; and risk monitoring.
Risk monitoring	Process of following up the decisions and actions within risk management in order to ascertain that risk containment or reduction with respect to a particular hazard is assured. Risk monitoring is an element of risk management.
Safety	Practical certainty that adverse effects will not result from exposure to an agent under defined circumstances. It is the reciprocal of risk.

Term	*Description*
Safety factor	Composite (reductive) factor by which an observed or estimated no-observed-adverse-effect level (NOAEL) is divided to arrive at a criterion or standard that is considered safe or without appreciable risk. Related terms: *Assessment factor, Uncertainty factor*
Threshold	Dose or exposure concentration of an agent below which a stated effect is not observed or expected to occur.
Tolerable daily intake	Analogous to *Acceptable daily intake*. The term "tolerable" is used for agents that are not deliberately added, such as contaminants in food.
Tolerable intake	Estimated maximum amount of an agent, expressed on a body mass basis, to which each individual in a (sub)population may be exposed over a specified period without appreciable risk.
Toxicity	Inherent property of an agent to cause an adverse biological effect.
Uncertainty	Imperfect knowledge concerning the present or future state of an organism, system, or (sub)population under consideration.
Uncertainty factor	Reductive factor by which an observed or estimated no-observed-adverse-effect level (NOAEL) is divided to arrive at a criterion or standard that is considered safe or without appreciable risk. Related terms: *Assessment factor, Safety factor*
Validation	Process by which the reliability and relevance of a particular approach, method, process, or assessment is established for a defined purpose. Different parties define "Reliability" as establishing the reproducibility of the outcome of the approach, method, process, or assessment over time. "Relevance" is defined as establishing the meaningfulness and usefulness of the approach, method, process, or assessment for the defined purpose.

Other terms used in this guidance.

Term	*Description*
Adjustment factor	A factor based on quantitative chemical-specific toxicokinetic or toxicodynamic data, which replaces the default uncertainty factor. The term has a similar meaning to assessment factor (see above table).
Benchmark dose/ concentration (BMD/BMC)	The dose/concentration, or the lower confidence limit of the dose/ concentration, calculated to be associated with a given incidence (e.g., 5% or 10% incidence) of effect estimated from all toxicity data on that effect within that study.

Term	*Description*
Categorical factor	A factor based on common characteristics of a group of compounds, e.g., physical/chemical properties or lowest-observed-adverse-effect level (LOAEL) to no-observed-adverse-effect level (NOAEL) correction.
Chemical-specific adjustment factor (CSAF)	A quantitative measurement or numerical parameter estimate that replaces a default uncertainty subfactor.
Composite uncertainty factor (CUF)	The product of any chemical-specific adjustment factors (CSAFs) and the residual default subfactors that were not replaced because of lack of relevant data.
Critical effect	The relevant adverse effect, or its known precursor, that is produced at the lowest dose or concentration on the dose/concentration scale.
Default value	Pragmatic, fixed or standard value used in the absence of relevant data.
Key event	Measurable events that are critical to the induction of tumours as hypothesized in the postulated mode of action.
Lowest-observed-adverse-effect level/concentration (LOAEL/ LOAEC)	The lowest concentration or amount of a substance, found by experiment or observation, that causes an adverse alteration of morphology, functional capacity, growth, development or life span of the target organisms that is distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.
Mechanism of action	Relates to sufficient understanding of the molecular basis to establish causality.
Metric	A relevant quantitative measurement or parameter estimate.
Mode of action	Evidence provided by robust mechanistic data to establish a biologically plausible explanation.
No-observed-adverse-effect level/concentration (NOAEL/ NOAEC)	The greatest concentration or amount of a substance, found by experiment or observation, that causes no detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organisms under defined conditions of exposure. Alterations may be detected that are judged not to be adverse.
Subfactor	The result of subdividing the default factor for either interspecies differences or human variability into separate values for toxicokinetics and toxico-dynamics, which, when multiplied, give the same numerical value as the original interspecies or human variability factor.

Term	*Description*
Toxicodynamics	The process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects (IPCS, 1994).
Toxicokinetics	The process of the uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of the substances and their metabolites in the tissues and the elimination of the substances and their metabolites from the body. Both the amounts and the concentrations of the substances and their metabolites are studied. The term has essentially the same meaning as pharmacokinetics, but the latter term should be restricted to the study of pharmaceutical substances (IPCS, 1994).
Uncertainty factor	A product of several single factors by which the NOAEL, NOAEC, LOAEL, LOAEC, BMD or BMC of the critical effect is divided to derive a tolerable intake. These factors account for adequacy of the pivotal study, interspecies extrapolation, interindividual variability in humans, adequacy of the overall database and nature of toxicity. The term uncertainty factor is considered to be a more appropriate expression than safety factor since it avoids the notion of absolute safety and because the size of this factor is proportional to the magnitude of uncertainty rather than safety. The choice of uncertainty factor should be based on the available scientific evidence.