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A Probabilistic Framework for the Reference Dose (Probabilistic RfD)

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Determining the probabilistic limits for the uncertainty factors used in the derivation of the Reference Dose (RfD) is an important step toward the goal of characterizing the risk of noncarcinogenic effects from exposure to environmental pollutants. If uncertainty factors are seen, individually, as "upper bounds" on the dose-scaling factor for sources of uncertainty, then determining comparable upper bounds for combinations of uncertainty factors can be accomplished by treating uncertainty factors as distributions, which can be combined by probabilistic techniques. This paper presents a conceptual approach to probabilistic uncertainty factors based on the definition and use of RfDs by the U.S. EPA. The approach does not attempt to distinguish one uncertainty factor from another based on empirical data or biological mechanisms but rather uses a simple displaced lognormal distribution as a generic representation of all uncertainty factors. Monte Carlo analyses show that the upper bounds for combinations of this distribution can vary by factors of two to four when compared to the fixed-value uncertainty factor approach. The probabilistic approach is demonstrated in the comparison of Hazard Quotients based on RfDs with differing number of uncertainty factors.

KEY WORDS: Probabilistic; uncertainty factor; distribution; reference dose; hazard quotient.

1. INTRODUCTION

Establishing exposure levels of a substance at or below which there is a minimal risk of adverse health effects is the basis of the current system for managing noncarcinogenic risks from exposures to chemicals in the environment. The establishment of plausible limits on these exposure levels is an important goal for improving the credibility of noncancer risk assessment in general. One specific step toward this goal is to analyze the uncertainties in the key components of the Reference Dose (RfD), the standard tool used by the U.S. Environmental Protection Agency (U.S. EPA) to estimate risks for the noncarcinogenic effects of chemicals.[1,2] A number of issues have been raised concerning the RfD and the current system for evaluating noncarcinogenic risks.[3–19] In particular, the RfD relies on the use of a series of uncertainty factors, each of which is conservative (with respect to protection of public health). The result is the inability to compare RfDs that use different numbers of uncertainty factors relative to the degree of protection provided. The RfD also depends on the establishment of a no-observed-adverse-effect level (NOAEL), which is dependent on study design factors that are not consistent across studies.[3–13] In addition, the current approach for deriving RfDs does not provide the risk manager with insight concerning the potential hazard posed by a chemical when exposures exceed the RfD.[12] Instead the risk manager is given a limit below which an appreciable risk is thought to be absent. Finally, the quantification of RfDs is driven largely by the uncertainty associated with limited toxicological infor-

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Table I. Uncertainty Factors and Associated Extrapolations Across Endpoints

Uncertainty factor	Estimated endpoint	Measured endpoint
Interindividual (UF_H)	NOAEL in a sensitive subpopulation	NOAEL in the general population
Interspecies (UF_A)	NOAEL in a typically healthy human population	NOAEL in a test species
Subchronic (UF_S)		NOAEL in a subchronic study
LOAEL (UF_L)		LOAEL in a study
Database (UF_D)	The lowest NOAEL observed in a set of toxicological studies	NOAEL in a chronic study

mation, yet little guidance is provided for evaluating the uncertainty. Understanding this uncertainty is critical to risk managers who are required to evaluate risks to individuals whose exposures exceed the RfD.

This paper presents an approach for a probabilistic interpretation of RfDs in the context of the current definition of the RfD. The presentation begins with a redefinition of the RfD in the operational sense and the development of a conceptual framework for defining the current uncertainty factors within the context of the operational definition. Next, a generic "reference" distribution for the uncertainty factors is derived that takes into account the definition and practice of the RfD methodology, but does not necessarily consider either the underlying biological mechanisms or empirical data that might be used to define specific uncertainty factors. The reference distribution is then used to explore the probabilistic implications in the use of uncertainty factors. Finally, a discussion is presented on the interpretation of the reference distribution with respect to theoretical considerations and empirical information.

2. REDEFINING THE RfD

The RfD currently is defined by the U.S. EPA[1] as

> . . .an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime.

RfDs are derived using the formula given in Eq. (1).[1,2]

$$RfD = \frac{\text{NOAEL}}{\text{UF} \times \text{MF}} \tag{1}$$

where the NOAEL is the no-observed-adverse-effect level in mg substance per kg body weight per day (mg/kg-d), UF is a composite uncertainty factor comprising multiple individual uncertainty factors and MF is a situation-specific modifying factor. Historically, point estimates have been used to establish RfDs. That is, a single value, with perhaps one or two significant figures, has been used as a measure of the NOAEL or LOAEL and each of the uncertainty factors in the formula used to derive RfDs.[1,2] Table I presents the uncertainty factors and their associated endpoints.

A historical perspective of the RfD is presented elsewhere.[20] Of primary interest, here, is the treatment of uncertainty factors as distributions. Dourson and Stara[21] provide an empirical perspective for the uncertainty factors, defining them in terms of plausible "limits" on the distribution of the ratios of doses associated with the endpoints defining each area of uncertainty. Within the context of such an empirical framework, Baird *et al.*[19] provide probabilistic RfD estimates for specific substances based on empirically-derived uncertainty factor distributions. Price *et al.*[22] present probabilistic implications for evaluating noncarcinogenic risks above the RfD using empirical and theoretical uncertainty factor distributions. The derivation of specific empirical distributions, however, is not necessary as a first step in the definition of uncertainty factor distributions. A useful probabilistic description of uncertainty factors can be inferred from the definition of the RfD.

With reference to the definition of the RfD given at the beginning of this section, the imprecision arises from the fact that the RfD is based on *observed* no effect levels and, except for the case where UF = 1, on one or more uncertain extrapolations. That particular case, however, allows for a more tangible definition of the RfD. The total quantified uncertainty is reduced to unity (UF = 1) when a NOAEL in a sensitive human subpopulation ($NOAEL_{HS}$) has been identified, as it has been for the nitrate and fluoride RfDs.[24] This consideration leads to the definition of the RfD as an estimate of the $NOAEL_{HS}$. That is, the "reference" in the RfD is not a specific point on any dose-response curve but is the NOAEL in a "sensitive" human study. As currently derived, however, the RfD is a biased estimator of the $NOAEL_{HS}$ because of the use of multiplicative uncertainty factors. As the RfD is designed to be protective

and can be based on one uncertainty factor, each uncertainty factor necessarily is protective alone. This results in a consideration of the RfD (for UF $>$ 1) as a sort of lower confidence limit on the estimate of the $NOAEL_{HS}$. As will be shown subsequently, the probability associated with this "confidence limit" varies with the number of uncertainty factors used.

3. THE UNCERTAINTY IN THE TOXICOLOGICAL ESTIMATES USED IN SETTING THE RfD

The NOAEL is the highest of the tested doses[4] in a toxicological experiment that is judged not to have caused an adverse effect. The NOAEL is often taken as a surrogate for a threshold, or something near a threshold. The NOAEL, however, is dependent on the determination of the LOAEL by statistical or biological significance and is thus dependent on the power of the study to detect an effect. This dependence results in a bias of the NOAEL as an estimator of a threshold.[3–13] This bias is implicitly accepted in the RfD methodology when it pertains directly to a studied sensitive human subpopulation (the $NOAEL_{HS}$), in which case the quantified uncertainty is reduced to unity. The $NOAEL_{HS}$, then, is that exposure "likely to be without appreciable risk of deleterious effects during a lifetime," or, in other terms, a "minimal risk level." This description implies a certain amount of residual risk at the NOAEL, which is not consistent from one study to another because of differences in study sensitivity. The uncertainty in the NOAEL, itself, is related to what the NOAEL might have been had the study been optimally designed and conducted. That is, the uncertainty in the NOAEL relates to differences in residual risk at the NOAEL across studies. The probabilistic approach presented in this paper does not address this particular uncertainty but, rather, follows the RfD methodology in accepting the $NOAEL_{HS}$ as the endpoint for protection of human health.

4. THE UNCERTAINTY IN THE UNCERTAINTY FACTORS

In this paper we have adopted the approach established by Dourson[20] that views uncertainty factors as approximate upper bound estimates for the uncertainty for each step of the process of extrapolating from available toxicological data to a dose that is protective of sensitive individuals (the $NOAEL_{HS}$). This process occurs by determining values for a series of surrogate toxicological measurements, such as a chronic NOAEL in test animals or a chronic NOAEL in typical healthy humans. For any given compound the ratios between these surrogate toxicological measurements are a series of fixed values. The uncertainty associated with such ratios for an untested chemical can be investigated by assuming that the chemical is a random member of a universe of chemicals. In this case, the universe comprises all chemicals whose suspected toxicity warrants testing. The uncertainty in the value for the individual chemical is represented by the variability across the population of all such chemicals.[25,26] The shape of this distribution of ratios can be estimated from the distribution of ratios observed in a sample of known chemicals. Distributions of ratios determined in this fashion include both interchemical variation and study design variability. That is, the uncertainty in the NOAEL is aggregated with interchemical variability. A description of the basis and plausible range for each uncertainty factor follows.

4.1. Interspecies Uncertainty (UF_A)

Interspecies uncertainty refers to the uncertainty associated with using laboratory animal toxicology studies to predict NOAELs in the general human population. Specifically, UF_A is the ratio of the NOAEL in a chronic laboratory animal study to the (putative) NOAEL in a human study that did not include a significant number of members from the sensitive subpopulation. The uncertainty in UF_A arises from species-related differences in toxicokinetics (metabolic processes of absorption, distribution, biotransformation and elimination)[27] and toxicodynamics (biochemical and physiological effects and mechanisms of action).[28] That is, UF_A aggregates the cross-species variability in the processes that determine the fate and transport of the substance in the organism and in the ultimate target-organ sensitivities.

The data needed to define UF_A are studies in the general human population paired with laboratory animal studies for exposures to the same toxic agent. These types of comparisons, however, are relatively rare in the literature. One indirect approach for defining UF_A assumes an allometric relationship for toxicity across species and uses the observed variability around that relationship for several test animal species[5] to estimate

[4] The term, "dose," as used in this paper actually represents a dose rate, as in mg/kg-day.

[5] For a summary of the pertinent literature see Refs. 29–32.

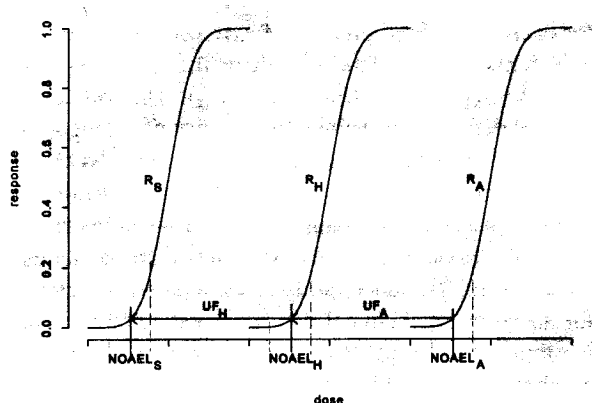

Fig. 1. Definition of UF_A and UF_H in terms of NOAELs in test animals and the general and sensitive human populations.

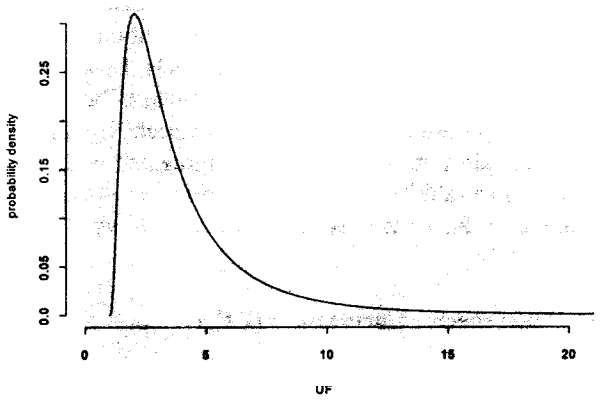

Fig. 2. Reference uncertainty factor distribution—UF_R.

the uncertainty in the allometric relationship for humans.[19] Another possibility is to use, as a surrogate, other endpoints that have been more commonly measured for both humans and test animals, such as pharmacokinetic end points.[33,34] The use of surrogates always introduces some unquantifiable uncertainty as to the accuracy of the representation.

Assuming that there is an allometric component in the toxic response across species, then toxic doses in laboratory animals (with lower body weights) will tend to underestimate the toxicity in humans.[21,34] That is, when doses are expressed on a mg/kg basis, UF_A would tend toward values greater than one. From a toxicodynamic perspective, however, laboratory animals are not necessary less sensitive than humans,[30,33] suggesting that UF_A can take on values less than 1.

4.2. Interindividual Uncertainty (UF_H)

Interindividual uncertainty refers to the variation in sensitivity among the members of the human population. UF_H specifically accounts for the uncertainty in estimating $NOAEL_{HS}$ based on a NOAEL in an average healthy population of humans. Because of the large heterogeneity in the human population, the finding that a compound does not cause adverse effects at a specified dose in a specific population of humans as identified in an epidemiologic or occupational-health study does not establish that the dose is without risk to some sensitive subpopulation of humans not included in the study population. Such sensitive subpopulations may include the fetus, the very young, the very old and individuals with predisposing conditions arising from genetic variation, disease, or dietary variation or deficiency.

The data needed to define UF_H are studies in the general human population paired with studies that include the presumed sensitive human subpopulation for exposures to the same toxic agent. Indirect approaches for the quantitative definition of UF_H include using the universe of test animals as a surrogate for humans[21] or using human interindividual pharmacokinetic variability as a surrogate for human variability in susceptibility.[35] The former assumes that the heterogeneity represented by combined test species would approximate human heterogeneity and the latter that susceptibility is largely a function of delivery of the toxin to the target tissue. Both assumptions have limitations that preclude the use of either of these approaches as surrogates for UF_H by themselves.

While interindividual variation has been studied by a number of researchers,[17,35,36] there is limited information directly applicable to the determination of either the median or upper limit for UF_H. Other characteristics of the distribution, however, can be conceptualized. First, the lower bound is one (1) by definition. Second, the subpopulation that UF_H addresses is limited to the fraction of individuals responding at or below the NOAEL in a study of the general human population response. Figure 1 is a graphical representation of UF_A and UF_H with respect to hypothetical dose-response curves for sensitive humans (R_S) "average healthy" humans (R_H) and test animals (R_A). $NOAEL_S$ is equivalent to $NOAEL_{HS}$ as defined previously. R_A is interpreted as the composite of all potential laboratory test species because $NOAEL_A$ is defined as the NOAEL in the most sensitive available study irrespective of the test species.[1,2] Figure 2 represents UF_A as the ratio of the NOAEL in test animals to an equivalent response level in humans, such as the NOAEL determined from an epidemiologic or occupational study of average healthy individuals. UF_H, then, must only account for the response between $NOAEL_{HS}$ and the residual population risk at $NOAEL_{HS}$. The population risk (based solely on limitations of sam-

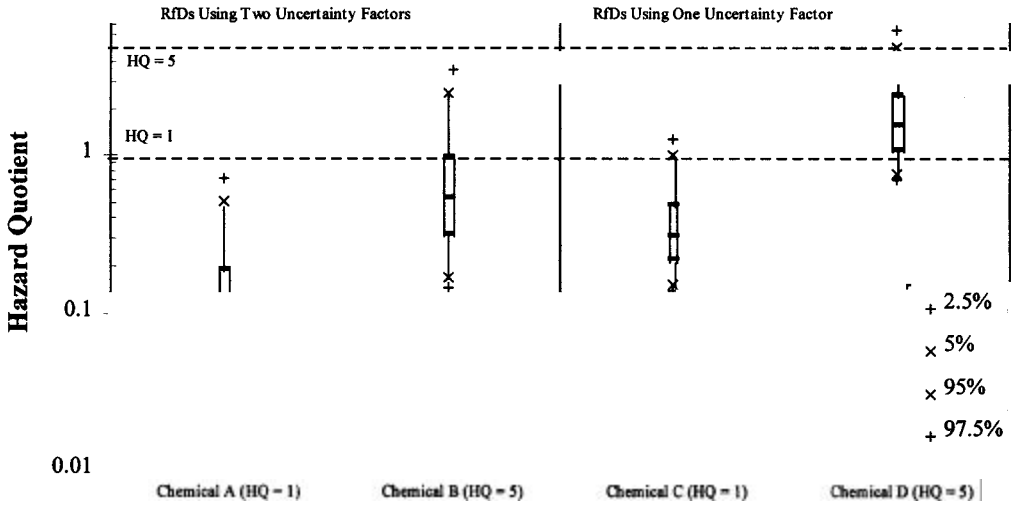

Fig. 3. Uncertainty in hazard quotients case study.

ple size) at the $NOAEL_{HS}$ has been interpreted as around 3%,[19] but could range from zero to 20% or more.[9,10] The location of the $NOAEL_A$ on the dose-response curve (R_A) is dependent on the sensitivity[6] of the study from which the NOAEL is determined relative to the universe of such studies (that is, for a complete data base; see section 4.5 following). The exact response interpretation of any NOAEL is, of course, uncertain. In particular, $NOAEL_{HS}$ depends on how well the sensitive subpopulation has been defined. In most cases, a specific subpopulation cannot be identified.

4.3. Subchronic to Chronic Uncertainty (UF_S)

The distribution for UF_S is the frequency distribution of the ratio of the subchronic NOAEL to the chronic NOAEL for all substances. The empirical data required to establish this distribution are NOAELs from subchronic and chronic studies for specific substances. The expected value of UF_S is greater than one as the chronic NOAEL is expected to be less than the subchronic NOAEL[19,21,37–39] presumably as a result of continuing insult resulting in unrepaired damage. The plausible lower bound for UF_S is 1 (although development of tolerance to the substance beyond subchronic exposure could result in dose ratios of less than 1). Within the current RfD methodology, UF_S does not consider differences among species, endpoints, or severity of effects; the same factor is applied in all cases. Also, although exposure duration is an inherently continuous variable, only one type of extrapolation, subchronic-to-chronic, is recognized.

[6] Study sensitivity is operationally determined by the dose level at which adverse effects appear. That is, the most sensitive study is the one with the lowest LOAEL.

4.4. LOAEL to NOAEL Uncertainty (UF_L)

UF_L is used when the lowest dose tested is an adverse-effect level (AEL). That is, a NOAEL has not been defined resulting in the use of a LOAEL in the numerator of Eq. (1). UF_L can be thought of as a dose-scaling factor for estimating what the NOAEL might have been had lower doses been tested. The distribution for UF_L is the frequency distribution for all substances of the ratio of a LOAEL to a putative NOAEL when the latter is lacking. The absence of a lower bound on the LOAEL means that UF_L must take into account any dose in the dose-response continuum that could be judged to be an AEL. There is no assumption that, had the NOAEL-less study been more fortunately designed, the NOAEL would be the next lower dose level. That is, the likelihood that the next lower dose level would be a NOAEL is unknown. UF_L, then, is dependent on the placement of the LOAEL in the dose-response continuum, which, in turn, is dependent on the incidence and severity of effects and on the slope of the dose-response curve. Common practice in the application of UF_L is to apply a factor between 1 and 10 depending on the incidence and judged severity of the observed effects.[1,24]

The UF_L can be inferred from the severity of the observed effects and the history of AEL:NOAEL ratios

from studies of other substances. The data required to establish the distribution for a generic UF_L are studies showing the full range of effects from no effects to "frank" effects. Frank effects, in this case, are defined as those that would normally be considered too severe for the basis of an RfD[1] and establish the upper bound for an AEL; the NOAEL establishes the lower bound. That is, the ratio of the frank-effect level (FEL) to the NOAEL is the upper limit for UF_L. The lower bound for UF_L is asymptotic to 1 by definition because the NOAEL must be less than the LOAEL. As an alternate approach, UF_L could be determined from the dose-response information available for the substance in question. As an example, the benchmark dose method[6] could be used to estimate a NOAEL "equivalent" as an alternative to an uncertainty factor.

4.5. Database Adequacy Uncertainty (UF_D)

The distribution for UF_D is the frequency distribution of the ratio of a chronic NOAEL (directly observed or estimated from a subchronic study) to the NOAEL from a complete database for all substances. The complete database within the context of the RfD methodology is defined as chronic toxicity studies in two species (one nonrodent), a multigeneration reproduction study, and developmental toxicity studies in two species.[20,40] UF_D is actually a family of distributions, with a separate distribution required for each combination of studies that might arise. Complete data sets for individual substances are required to establish these distributions empirically.[21] As a simplification, only the case where a single chronic NOAEL (or chronic NOAEL estimate) is available will be used in this paper; this is the maximum uncertainty scenario where $UF_D = 10$.

An issue not fully addressed in the RfD methodology is that the overall NOAEL for the database is based on the most sensitive study irrespective of the number of studies available beyond that required for a complete database. Additional studies cannot reduce the size of the uncertainty factor; they can only lower the overall NOAEL and, hence, the RfD. The RfD methodology does allow for the use of a modifying factor of less than 1 but there is little guidance for this situation and it has never been done in practice.

5. PROPOSED APPROACH

As described in Section 2, this manuscript addresses the RfD in an "operational" context rather than attempting to redefine the RfD in terms of specific levels of risk. The focus of the approach presented here is the presentation of the probabilistic implications of uncertainty factors as they are currently defined and applied rather than in a mechanistic or empirical context. In this context, a single generic "reference" distribution is used to characterize the uncertainty associated with each of the current factors. This distribution is based upon the interpretation that an uncertainty factor of 10 is conservative (protective) with respect to risk, recognizing that current assumptions are not necessarily consistent with empirical toxicological findings. The latter is an inherent limitation to both the RfD methodology as practiced today and the probabilistic approach presented here. The development of empirically-based characterizations of uncertainty for each of the existing uncertainty factors is necessary for establishing any sort of accuracy of a probabilistic approach. An empirical approach is presented by Baird *et al.*[19] which may prove to be a useful start in this direction. As empirically-derived distributions have not yet been adopted by consensus, assessments based on reference distributions can prove useful as a means to evaluate the *relative* magnitude of uncertainty for RfDs based on different numbers of uncertainty factors. The current system of uncertainty factors does reflect a consensus on the magnitude of the uncertainty associated with the establishment of an RfD. As a result, the results of this analysis can be viewed as an extension of the current methodology for evaluating noncarcinogenic risks that is subject to amendment when adequate data become available.

5.1. The Use of Monte Carlo Methods to Estimate the Total Uncertainty in an RfD

The approach presented in this paper interprets the variables in Eq. (1) as distributions of values rather than as a point estimates. The distribution assigned to each variable, or input, reflects the uncertainty in its value. As a reflection of this consideration and of the operational definition of the RfD proposed previously, the RfD formula is rewritten as:

$$\mathrm{NOAEL}_{HS} = \frac{\mathrm{NOAEL}_{exp}}{\Pi U_i} \qquad (2)$$

where NOAEL_{exp} is the experimental NOAEL and ΠU_i is the product of the individual uncertainty and modifying factors required for each substance. In a full analysis, each of the inputs in Eq. (2) would be replaced by specific PDFs that characterize their respective uncertainty; the PDFs express the probability that the input

has a specified value. An output distribution for the $NOAEL_{HS}$ then would be estimated by means of Monte Carlo analysis.[41,42] The uncertainty distributions express the uncertainty in each step in the process of extrapolating from the measured toxicological endpoint to the final RfD value, which is the lower confidence limit on the expression in Eq. (2). The overall uncertainty in an RfD for a specific compound arises from the uncertainty in each of the U_i and the uncertainty in the NOAEL. The MF is not considered here, as it is always situation-specific and no general representation exists. The uncertainty in the numerator of Eq. (2) can be considered when a suitable model for the NOAEL is developed. The Monte Carlo analyses are performed only for the denominator of Eq. (2).

5.2. Development of a "Reference" Distribution for Uncertainty Factors

The generic, or "reference," distribution is based, primarily, on the probabilistic implications of the conceptual definition[1,2] and commonly-used uncertainty factor values.[24] That is, this approach attempts to minimize assumptions that cannot be reasonably justified within the context of the current RfD methodology. Thus, although certain of the uncertainty factors can conceptually take on values less than 1, the approach taken in this paper follows the RfD methodology, which does not allow values of less than 1 for any uncertainty factor. The impact of any additional assumption on the RfD uncertainty is investigated by performing a sensitivity analysis.

5.3. Derivation of the Reference Distribution

There is no distinction in the current RfD methodology as to the relative quantitative importance of any given uncertainty factor, as each has a nominal value of 10 and a minimum value of 1. A single "reference" uncertainty factor distribution (U_R), therefore, is used to represent the uncertainty in each of the five factors. A primary assumption is made that the natural variability underlying each of the uncertainty factors is a result of many multiplicative factors. Variables arising from such processes will tend to be lognormally distributed and products or ratios of lognormal distributions will, in turn be lognormally distributed.[43] This assumption is implied in the definition of the RfD ("...with uncertainty spanning perhaps an order of magnitude...") and by the common use of $10^{0.5}$ (3.16, rounded to 3) as the standard alternate uncertainty factor value when 10 is considered too high. The assumption is also consistent with the traditional practice of using the log-transformed dose in dose response modeling.

A distribution that satisfies these assumptions is a three-parameter lognormal.[44] The three-parameter lognormal is a standard two-parameter lognormal that is shifted to the left or right on the x-axis, starting at a value other than zero. In this case, the distribution starts at one. The parameters of the three-parameter lognormal distribution are the mean (μ), the standard deviation (σ) and the offset (τ). In this case, τ is equal to one. The more commonly used two-parameter lognormal distribution corresponds to $\tau = 0$. The parameters are set such that the median (50th percentile) is $10^{0.5}$ and the 95th percentile is 10 [$Pr(U_i \leq 10) = 0.95$]. The latter assignment is based on the concept that RfDs are designed to be protective[7] and can be based on a single uncertainty factor. The nominal value of 10, therefore, represents a "high-end" estimate of the uncertainty for any given uncertainty factor. "High end" is interpreted in the context of the phrase in the definition of the RfD, "...unlikely to result in...," as similar to an upper confidence limit on the uncertainty factor, but not the absolute maximum. Setting the 95th percentile at 10 means that the expectation is that the actual reduction in the NOAEL will be greater than 10 in 5% of the cases when the missing data are supplied. The choice of $10^{0.5}$ for the median is based on the common use of the value of 3 ($10^{0.5}$ rounded to one digit) as an alternate uncertainty factor[24] and limited empirical support.[21,37] Any choice of percentile is inherently arbitrary and is only meant to serve as a point of reference for comparisons of multiplicative combinations of uncertainty factors. As a convention μ and σ will be expressed in terms of the underlying normal distribution of the logarithms to the base 10 ($\log_{10}$) of the corresponding U_i value. For the three-parameter lognormal distribution, μ is equal to the logarithm of the offset-adjusted median of U_R [$\mu = \log_{10}(\text{median}(U_R) - \tau)$]. The parameter values satisfying the assumptions are $\mu = 0.335$ and $\sigma = 0.3765$. The reference uncertainty factor distribution, U_R, is shown in Fig. 2.

[7] That is, it is unlikely to result in appreciable risk of adverse effects in sensitive humans.

Table II. Selected Percentiles for Combinations of U_R[a]: $Pr(U_R \leq 10) = 0.95$

Percentile	U_R	U_R^2	U_R^3	U_R^4	U_R^5
50	3.16	11	37	127	433
95	10.0	51	234	1,040	4,440
99	17.3	104	544	2,700	12,700

[a] Three-parameter lognormal ($\mu = 0.3349$, $\sigma = 0.3765$, $\tau = 1$).

5.4. The Use of the Reference Distribution to Characterize the Uncertainty in RfDs with Varying Number of Uncertainty Factors

An independent instance of U_R is invoked for each U_i in Eq. (2) applicable to a given RfD scenario. That is, a separate and independent random iteration of U_R is substituted for each U_i in the denominator of the RfD formula (Eq. 2) for each of the N iterations of the Monte Carlo simulation and the equation is solved. The result is an output distribution of N independent composite uncertainty factor (UF) estimates. The output distribution can be used to determine the likelihood of any specific UF value that would be obtained should the complete data be available. Separate simulations are performed for two, three, four, or five uncertainty factors. The results of each simulation, expressed as selected percentiles of the output distribution, represent the average of ten independent runs of 100,000 iterations each. All simulations were performed in S-PLUS® (Version 3.2) for Windows® (Version 3.1).

UF values at selected percentiles for each Monte Carlo simulation for the three-parameter U_R are given in Table II. The values in Table II are meant to be compared to the standard (in the current RfD methodology)[20] composite UF values of 100, 1,000, 3,000 and 10,000 for combinations of two (U_R^2), three (U_R^3), four (U_R^4), and five (U_R^5) uncertainty factors, respectively. Table II shows that, with the exception of U_R^3, the standard UF values fall near the 99th percentile of their respective distributions and are probabilistic equivalents for their respective scenarios. The 99th percentile for U_R^3 is about half the standard value of 1000.

U_R is intended to be used only for full tenfold uncertainty factors. If, for a particular RfD, the data warrant a reduced uncertainty factor, such as for UF_D when only a reproductive study is missing, or for UF_S when the exposure duration is intermediate between subchronic and chronic, U_R does not apply. In these cases, an uncertainty factor of three (3) often will be used to reflect conditions of reduced uncertainty,[8] but still would be interpreted as a loose upper bound on the uncertainty. One choice for a distribution would be "half" of U_R (median of $10^{0.25}$ and 95th percentile of $10^{0.5}$). A simple approximation would be the square root of U_R.

5.5. Effect of the Form of the Input Distribution and Choice of Distribution Parameters on the Output

As a means of determining the sensitivity of U_R to the form of the distribution, parameters are also defined for the two-parameter lognormal, log triangular, log beta and log logistic distributions. The quantitative assumptions are the same as for the three-parameter lognormal distribution. That is, the parameters of the alternate distributions are selected such that the 0th, 50th and 95th percentiles are $1, 10^{0.5}$ and 10, respectively. The Monte Carlo simulation results show only a small effect of the form of the input distribution on the output. The simulation values vary within a range of 11% for the 95th percentile and 28% for the 99th percentile. The assumption for $Pr(U_R \leq 10)$ also has relatively little impact on the results. Varying $Pr(U_R \leq 10)$ from 0.90 to 0.99 results in a twofold range for the 95th percentile of a single U_i, but only a 4–18% change in the relative U_R" simulation output at the $Pr(U_R \leq 10)$ percentile. That is, the simulation values at the 90th percentile for $Pr(U_R \leq 10) = 0.90$ or for the 99th percentile for $Pr(U_R \leq 10) = 0.99$ are very close to the simulation values at the 95th percentile for $Pr(U_R \leq 10) = 0.95$. Varying the median over a twofold range (2.0–4.0) has a much greater impact on the output than changes in $Pr(U_R \leq 10)$, resulting in up to a threefold change in U_R^5 at the 95th percentile.

6. IMPLICATIONS FOR THE EVALUATION OF THE POTENTIAL FOR ADVERSE NONCARCINOGENIC EFFECTS

The current system of evaluating noncarcinogenic risk is essentially a comparison of the estimated dose to the RfD.[45] Such a comparison is used by risk managers to ascertain whether the exposure is above a dose which is unlikely to result in adverse or deleterious effects (a dose less than the RfD) or one judged to have some potential to cause an adverse effect (a dose greater than

[8] These situations are not the same as when a factor of 3 is used for one area of uncertainty simply to reduce the "conservatism" in the total UF (i.e., otherwise, no data suggesting reduced uncertainty for any specific area of uncertainty).

the RfD). The comparison of the dose and the RfD is expressed in terms of a Hazard Quotient (HQ).[45] The HQ is defined as the ratio of the dose resulting from exposure to a single chemical to the RfD as in Eq. (3).

$$HQ = \frac{D_i}{RfD_i} \quad (3)$$

where D_i is the dose of chemical i and RfD_i is the RfD for chemical i. Regardless of the specific value of the RfD, the HQ ratio is designed to provide a common measure of relative risk across chemicals and exposure scenarios. This approach is intended to provide consistency for risk managers faced with evaluating exposures involving different chemicals with different toxicities.

The uncertainty in the HQ_i can be quantified by replacing RfD with $NOAEL_{HS}$ as calculated using Eq. (4):

$$HQ_i = \frac{D_i \, \Pi U_i}{NOAEL_i} \quad (4)$$

where $NOAEL_i$ is the NOAEL for chemical i. The uncertainty in the estimate of HQ_i can now be modeled using Monte Carlo analysis. As Eq. (4) indicates, the uncertainty in HQ_i is a function of the number of uncertainty factors used in its derivation As a result, HQs with the same RfD_i and D_i but different numbers of uncertainty factors will have different uncertainty distributions. To explore this issue, the uncertainty in the estimates of HQ was calculated for four different compounds.

In this case study, four different chemicals, A through D, are examined. The RfDs for chemicals A and B are established with two uncertainty factors. The dose for chemical A is associated with an HQ of 1 and the dose for chemical B is associated with an HQ of 5. The doses for chemicals C and D also have HQs of 1 and 5, respectively. The RfDs for chemicals C and D, however, were derived using a single uncertainty factor and are thus more "certain" than for chemicals A and B.

Figure 1 is a box-and-whiskers plot of the results obtained for these four chemicals. The graphs present the mean and 2.5th, 5th, 25th, 50th, 75th, 95th, and 97.5th percentiles. These graphs depict the uncertainty in the HQs for each of the chemicals. The probability that HQ_i is greater than 1 can be thought of as equivalent to the probability that the dose, D_i, is greater than the (unknown) $NOAEL_{HS}$ for chemical i. For chemicals A and B (two uncertainty factors each), the probabilities are 2.5% and 25%, respectively. That is, despite the HQ estimate of five, there is only a 25% probability that the dose of chemical B exceeds the $NOAEL_{HS}$. As the RfDs for chemicals C and D are more certain (one uncertainty factor each), there are higher probabilities that the doses exceed the $NOAEL_{HS}$. For chemical C there is a 5% chance that the dose could be greater than the $NOAEL_{HS}$, and for chemical D there is a 75% chance that the dose is greater than the $NOAEL_{HS}$.

7. DISCUSSION

The approach presented in this paper is intended to be consistent with the current definition and application of the RfD methodology keeping the number of additional assumptions to a minimum. Two critical assumptions for the RfD methodology, whether probabilistic or not, are that all NOAELs "are created equal" and that all U_i contribute equally to the overall uncertainty. Additional assumptions are made for the probabilistic approach presented in this paper about the nature of the distribution of the dose ratios comprising the U_i. Specifically, the U_i are assumed to be lognormally distributed with a minimum of 1, a median of $10^{0.5}$ and a 95th percentile of 10.

The first assumption (NOAEL equivalency) is probably valid only for the well-designed, well-conducted toxicological studies assumed to be the basis for the current uncertainty factors.[46] As discussed in Section 3, the uncertainty in the NOAEL has to do with the relative magnitude of residual risk at the NOAEL in different studies, which can vary greatly depending on the "sensitivity" of the study. Finding an alternative for the NOAEL is critical for distinguishing among NOAELs that vary greatly in quality. In the standard RfD methodology, then, uncertainty in the NOAEL is assumed implicitly to be negligible with respect to the uncertainty factors, themselves, or subsumed within the uncertainty factors.

As to the equivalence of the U_i, There are a number of conceptual quantitative and qualitative differences among the uncertainty factors such that all U_i are probably not equal. Differences in the lower bound have already been mentioned. In particular, UF_A potentially can take on values below 1. There are also conceptual differences in the probability densities in the vicinity of the lower bound for those uncertainty factors with an absolute lower limit of one. As an example, the UF_H probability density would be expected to be increasing from zero at $UF_H = 1$ to a maximum at the mode. The probability density for UF_D would be highest at 1, decreasing monotonically for higher U_i values.[40] UF_S, which has only a theoretical lower bound of I, is expected to have a finite probability density at 1 as there is no *a priori*

expectation that continuing exposure *must* lead to lower toxic doses. That is, UF_S would have higher probability densities closer to 1 than UF_H.

The assumption about the mathematical form of the distribution for U_R does not have a great impact on the output. The lack of sensitivity of the Monte Carlo simulation output to the form of U_R is not particularly surprising given the fixed anchors at the 50th and 95th percentiles and the general similarity of the shapes of the distributions. If a uniform distribution is assumed for U_R or if U_R is assumed to be distributed as a function of the dose ratios, rather than the log of the ratios, the upper quantiles of the simulation output would be much higher. Neither of these alternate assumptions, however, is consistent with the concept and use of uncertainty factors. Also, although the choice of $Pr(UF_R \leq 10)$ does not have an effect on the interpretation of the output, the choice of median does. As an example, for all assumptions of $Pr(UF_R \leq 10)$, the corresponding value for four uncertainty factors is close to 1000 but varies by more than 50% in either direction when the median ranges from 2 to 4. The specific choice for the median is the assumption least supported by the existing RfD methodology but should be easier to establish empirically than the extremes of the uncertainty factor distributions.

Although U_R is intended to represent a plausible estimate of the range of uncertainty, it may not adequately address the uncertainty in extreme values of the dose ratios comprising each of the specific areas of uncertainty. Of particular interest in the protection of public health is the possibility of catastrophic exceptions to any narrowly prescribed predictive distributional approach. U_R allows for only a 1% probability of values greater than 17, a value which has been exceeded for UF_S by somewhat greater frequency in some datasets.[21,37,38] Higher values for UF_A plausibly could occur at a much greater frequency than allowed by U_R, particularly for the smaller test species if an allometric relationship was assumed.[19] On the other hand, a factor of 10 may be adequate for protection of sensitive subpopulations given that UF_H must only account for those individuals responding below a NOAEL for the general population (see Fig. 1).

In the use of U_R for the probabilistic comparison of RfDs, one approach would be to set all composite uncertainty factors at the same probability level. At least two different implementations follow from different perceptions about the nature of uncertainty factors. If the degree of belief is high that the value of 10 is protective for each U_i alone, then the 95th percentile (corresponding to $U_i = 10$) of each of the combined uncertainty factor distributions should be used in the appropriate situation. The result would be a two to threefold reduction in the composite uncertainty factor for all uncertainty scenarios with more than one area of uncertainty (see Table II). If, however, the degree of belief is high that less than a 100-fold factor for laboratory animal studies is not protective, then the composite UF should be about the 99th percentile (corresponding to $U_R^2 = 100$) of the appropriate distribution. The composite uncertainty factors used in current practice are fairly consistent with the latter interpretation, with all but one close to the 99th percentile (Table II).

As demonstrated in Section 6, the use of probabilistic RfDs also provides an insight into the assessment of noncarcinogenic risks using Hazard Quotients. HQs of the same magnitude may represent different levels of concern depending on the number of uncertainty factors used in the derivation of the respective RfDs. This problem is likely to be compounded for the Hazard Index (HI) of mixtures, where the HQs of the individual components are summed.

8. CONCLUSIONS

The approach is not intended to be definitive nor for use in setting regulatory standards. The approach can be used to provide insight in the current process for evaluating noncarcinogenic risks. The primary practical value of this approach is for the comparison of RfDs for prioritization purposes. The probabilistic approach can be used to establish the RfD point estimate for applications that require an RfD as a variable in an equation or model, such as the HQ or HI. The use of probabilistic RfDs in formulas and models allows for the propagation of uncertainty through the model and into the result, ensuring that conservative assumptions are not repeated at each step. The use of probabilistic RfDs for including uncertainty in estimates of response rates is discussed in a related paper.[22] Uncertainty pertaining to qualitatively different NOAELs is a major remaining issue. In addition, empirical data need to be examined in order to establish more realistic uncertainty factor distributions.

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