Differential Sensitivity of Children and Adults to Chemical Toxicity

II. Risk and Regulation

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INTRODUCTION

Animals can be useful predictors of chemical hazards to humans. Growth and development are compressed into a shorter period in animals, which makes interpretation of animal testing inherently more difficult. However, similar events occur in both humans and laboratory animals and testing that covers the full period of animal development can reasonably be considered an appropriate surrogate for human development. Some have proposed an additional 10-fold factor for the extra protection of children when estimating safe exposures. Use of such an additional factor, as required by the Food Quality Protection Act (FQPA), is meant to address the same issues covered by the EPA’s database uncertainty factor, UF_D, and additional issues related to exposure uncertainty. Thus, when UF_D has already been deployed, the EPA modifies its use of the FQPA factor. Based on our analysis, we agree with the EPA. Drawing conclusions about the adequacy of UF_H, the uncertainty factor used to account for intrahuman variability, in terms of its ability to protect children on the basis of the modest data available is challenging. However, virtually all studies available suggest that a high percentage of the population, including children, is protected by using a 10-fold uncertainty factor for human variability or by using a 3.16-fold factor each for toxicokinetic and toxicodynamic variability. Based on specific comparisons for newborns, infants, children, adults, and those with severe disease, the population protected is between 60 and 100%, with the studies in larger populations that include sensitive individuals suggesting that the value is closer to 100%.

The process of identifying safe doses begins with the identification of no-observed-adverse-effect levels and knowledge of the qualitative and quantitative characteristics of chemical hazards. The qualitative nature of hazards can be characterized through appropriate toxicity testing using laboratory animals. Understanding age-related quantitative differences in sensitivity is more challenging.

To a large extent, the body of U.S. laws that seek to establish practices that will ensure safety—or at least mitigate risk—from chemical or other contaminant exposures provided the impetus for the development of methods to identify appropriate quantitative limits on chemical exposures. Most of the methods used today by regulatory agencies were developed in reaction to the calls by these laws to define limits on exposure that will “protect the public health with an adequate margin of safety” or lead to “a reasonable certainty of no harm.” That is, in passing the laws, the U.S. Congress called on the regulatory agencies to develop means to assess risks from chemicals or other agents so as to define exposure levels that would achieve the stated qualitative goals of health protection (Rhomberg, 1997).

Limiting exposures to chemical toxicants that achieve the public health goals set out by Congress often begins with the identification of “safe” or “virtually safe” doses. For example, the U.S. Food and Drug Administration (FDA) uses acceptable daily intakes to limit chemical exposures through food, the U.S. Occupational Safety and Health Administration uses permissible exposure levels to limit chemical exposures in the workplace, and the U.S. Environmental Protection Agency (EPA) uses reference doses (RfDs) and reference concentrations (RfCs)¹ to guide efforts to limit oral and inhalation exposures to chemicals, respectively.

¹ Please note that the majority of data that we discuss are for the oral route of exposure and, therefore, any conclusions that we draw should probably be restricted to this route. However, we anticipate that if sufficient inhalation data were available, similar conclusions might be drawn, especially if the critical effect is not limited to a portal of entry. We encourage additional analyses of inhalation data to test this supposition.
Differential Risk Between Children and Adults

TABLE 1
Description of Typical Uncertainty and Modifying Factors in Deriving Reference Doses (RfDs) or Reference Concentrations (RfCs)\(^a\)

<table>
<thead>
<tr>
<th>Standard Uncertainty Factors (UFs)</th>
<th>General guidelines(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H (interhuman)</td>
<td>Generally use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population.</td>
</tr>
<tr>
<td>A (laboratory animal to human)</td>
<td>For RfDs, generally use a 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. For RfCs, this factor is reduced to 3-fold when a NOAEL (HEC) is used as the basis of the estimate. In either case this factor is intended to account for the uncertainty in extrapolating animal data to humans.</td>
</tr>
<tr>
<td>S (subchronic to chronic)</td>
<td>Generally use a 10-fold factor when extrapolating from less than chronic results on experimental animals or humans. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs.</td>
</tr>
<tr>
<td>(LOAEL to NOAEL)</td>
<td>Generally use a 10-fold factor when deriving an RfD or RfC from a LOAEL, instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs.</td>
</tr>
<tr>
<td>D (incomplete data base to complete)</td>
<td>Generally use a 10-fold factor when extrapolating from valid results in experimental animals when the data are &quot;incomplete.&quot; This factor is intended to account for the inability of any single study to adequately address all possible adverse outcomes.</td>
</tr>
<tr>
<td>Modifying factor (MF)</td>
<td>Use professional judgment to determine an additional uncertainty factor termed a modifying factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and database not explicitly treated above (for example, the number of animals tested). The default value for the MF is 1.</td>
</tr>
</tbody>
</table>

Note. The maximum uncertainty factor used with the minimum confidence database for an RfD is 10,000; for an RfC it is 3000.


\(^b\) Professional judgment is required to determine the appropriate value to use for any given UF. The values listed in this table are nominal values that are frequently used by the EPA.

(NoAELs) or of doses that elicited a specific rate of response (benchmark dose, BMD), usually through laboratory animal testing. Laboratory animals are useful surrogates for humans, being similar in many respects. Humans may be more or less sensitive than laboratory animals. Laboratory animals are normally inbred and their responses to chemicals tend to be relatively uniform and consistent. For the purposes of regulation, humans are generally assumed to be more sensitive than the most sensitive species evaluated. Furthermore, permissible chemical exposure levels for humans must be safe for a variety of ethnic and otherwise dissimilar groups with inherently variable responses to chemical agents. In addition, laboratory animals are healthy and receive good nutrition; the same cannot be said for all people. For these reasons and for other special reasons described below, regulatory agencies have traditionally used "safety" or "uncertainty" factors to determine allowable levels for human exposure. Those levels are typically 100-fold or more below the doses that produce no adverse effect in the most sensitive laboratory animal studies.

The basic equation to determine a safe dose is

\[
\text{Safe dose} = \frac{\text{critical effect level}}{\text{uncertainty factor(s)}}
\]

Brief descriptions of commonly used safety or uncertainty factors (UFs) are provided in Table 1. These factors are reductions in the dose, based on scientific judgments of available toxicity, toxicodynamic, and toxicokinetic data and inherent uncertainty, necessary to identify an exposure level that is considered unlikely to produce adverse effects. The six specific categories shown in Table 1 follow the approach of the EPA. Although not all health organizations apply these factors as discrete divisors, most experts consider uncertainties in the following areas:

- extrapolation from shorter term exposures to longer term or lifetime exposures;
- absence of a NOAEL\(^2\);
- absence of adequate studies relevant to characterizing hazards;
- intrahuman variability;
- interspecies variability.

Several good reviews of this area are available (e.g., Dourson et al., 1996; Kalberlah and Schneider, 1998).

\(^2\) NOAEL: The highest exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse, nor precursors to adverse effects (EPA, 2000).
Concern about children’s potentially greater sensitivity to chemical toxicity is an important issue only in the context of potentially greater risk. Children are at greater risk of chemical toxicity if their exposures are high enough to produce adverse effects, whether they are more or less sensitive than adults. The question then is, are current regulatory approaches to limiting chemical exposures sufficient to protect children from exposures that are toxic; or, are chemical exposures misregulated due to inadequate attention to children’s sometimes greater sensitivity to particular chemicals, putting them at greater risk? This discussion generalizes the types of uncertainty listed above to answer the question of whether current regulatory approaches to determining safe chemical exposures are adequate to protect children. Specifically, it evaluates three questions:

- Is the uncertainty factor used to compensate for the absence of comprehensive toxicity testing adequate to protect children?
- Does the uncertainty factor used to account for the diverse sensitivity to toxicity among individuals protect children as well as adults?
- Are these two factors together adequate to protect children?

DETECTING AND CHARACTERIZING DEVELOPMENTAL HAZARDS

Toxicity testing plays a critical role in the detection and characterization of developmental hazards. Toxicity screening protocols can help flag potential developmental toxicants that should be subjected to further testing if widespread human exposure is likely. Further testing involves comprehensive protocols that have been developed to cover different periods of animal maturation in ways that are comparable to human development. For example, Fig. 1 shows a series of toxicity tests that covers most of the animals’ life stages.

Interspecies Temporal Concordance

Although infant and adult nonhuman animals differ in much the same way that human infants and adults differ, there are substantial interspecies differences among the young. For example, the newborn mouse or rat more nearly resembles the human fetus in the third trimester of gestation than the human infant at birth (NAS, 1983). However, the rates of maturation and growth of the mouse or rat after birth are much more rapid than those of the human infant. Maturity of a rat or mouse after weaning (6—8 weeks) does not appear to lag far behind the comparable time in the human infant (6—8 months). But, as indicated in Fig. 2, individual organ systems develop at different rates in different species. For example, as a percentage of mature weight, the human brain at 15—20 months of age is similar to the rat brain at 13—17 days of age (a roughly 30:1 temporal ratio) (Himwich, 1973).

The age–weight curves in Fig. 2 do not usually indicate functional maturity; most organs in the human are not fully mature functionally before they reach their final size. But a child or an animal at birth is reasonably well prepared for the abrupt changes that occur at parturition and most functional systems, although immature, possess a significant portion of their adult capacity. The growth curves also demonstrate a similar overall pattern of development among humans, mice, and rats based on physiological time.

Because of the different rates of maturation of specific functional systems in humans and animals, it is difficult to conduct cross-species temporal extrapolations between developing humans and developing animals. On the other hand, as long as the full course of development is tested in laboratory animals, there is every reason to believe that the same developmental processes occur sooner or later in humans and that the converse is also true. For most chemicals known to cause developmental effects in human, at least one animal species has been found to demonstrate similar effects (Hemminck and Vineis, 1985: Kimmel et al., 1990; Stanton and Spear, 1990).
FIG. 2. Organ development and stature or body weight as a percentage of adult values by age. Source. Based on data from Altman and Dittmer, 1962.

Developmental Toxicity Testing Protocols

Developmental toxicity testing protocols have been developed by national regulatory agencies and by other, multinational groups. Most of those protocols are similar and are designed to expose test animals in all the ways that humans may be exposed: prior to and during pregnancy and during nursing and postnatal life. Despite the comprehensive nature of such protocols and observed interspecies concordance, concerns have arisen over their adequacy and sensitivity due to the difficulty in fully understanding the similarities and disparities of important developmental milestones of the test species when compared with humans.

The embryos of mammals, including humans, are susceptible to common external influences, including nutritional deficiencies, intrauterine infections, mechanical problems, and chemical agents. Because of the rapid changes occurring during development, the nature and sensitivity of the embryo/fetus as a target for toxicity is also changing. The various developmental stages are relatively compressed in experimental animals. For example, the period of gestation is 21–22 days in the rat and 267 days in humans (Casarett and Doull, 2001).
Accordingly, the critical windows of vulnerability during the various stages of development occur earlier and are more closely spaced in experimental animals than in humans.

Table 2 compares a very rough timing of physiological maturation in rats and humans. It is apparent from this chart that, despite the approximations inherent in assuming comparable maturation rates of metabolic systems, exposing rats at 6 weeks or later does not include the most vulnerable periods of their development. The typical start time for rat studies, approximately 8 weeks, corresponds roughly to 12 years in the human infant, well after metabolic systems are fully developed. The 8-week start period for rat toxicity studies is largely a matter of practical convenience and feasibility. Rats and mice much younger than about 8 weeks are more difficult to handle and to subject to test protocols. The result is that the most vulnerable period in the child’s development is not directly evaluated by routine chronic and subchronic tests in animals. Nonetheless, chronic and subchronic tests have value in assessing potential risks to children by, for example, identifying target sites for toxicity and providing dose–response information that may be useful for human safety assessment, irrespective of life stage.

To compensate for the period of development not covered by routine chronic and subchronic toxicity testing, reproductive toxicity studies that expose the developing animal in utero have been used since the early 1940s. In these multigenerational studies, the parent animals (P0) are exposed through the period from weaning to mating, the P0 mothers are exposed through pregnancy and weaning, and their progeny (P1) are exposed until they mate. For the P1 offspring this protocol includes in utero exposure, exposure through mother’s milk and, after weaning, oral exposure through the diet. The new (F1) mothers are also exposed through their pregnancy and weaning. For the offspring of F1 parents, referred to as F2, this protocol also includes in utero exposure and exposure through their mother’s milk until weaning. Because of the immaturity of metabolic and physiologic systems, the first 6 months of the human infant period, corresponding approximately to 1–3 days in the rat, is the most critical from the viewpoint of potential developmental effects. Although the fetal stage is often considered the most vulnerable, the fetus appears to be adequately evaluated by standard developmental toxicity studies in two species and in two generations of the reproductive study. It is the immediate postnatal period that has garnered recent attention from toxicologists, because it is difficult to conduct extensive toxicity tests on newborn animals.

Christian (1986) reviewed 817 reproductive and developmental studies against rigorous criteria to evaluate the usefulness of results from multiple generations when compared to a single generation. Seventy-three studies passed the criteria, of which 38 reported positive results. Twenty of those positive studies showed effects that were more severe or first detected in the second or subsequent generations when compared to the first generation. These 20 studies were then critically reviewed to identify adult and litter primary reproductive effects and adult toxicity. The author concluded that if the objective of a reproductive study is to identify the lowest dosage producing a primary reproductive effect, one generation appears to be sufficient for evaluation. If evidence of bioaccumulation of the agent is evident, then more than one generation may be necessary to determine when the steady state of the agent is attained. It also appears that the evaluation of more than one litter per generation is not necessary to demonstrate a primary litter reproductive effect. Since the publication of this work, testing guidelines have been updated because of questions regarding the adequacy of older studies. It would be of interest to repeat this work on the basis of newer studies.

The United States, Japan, and the United Kingdom use a three-segment protocol for the evaluation of new pharmaceutical agents. Segment I is designed to evaluate fertility and general reproduction and to assess potential developmental effects in the offspring. Segment I is conducted using one species (usually the rat) and involves the treatment of males and females prior to conception and the continued treatment of females throughout gestation and lactation. Segment II is a teratology or developmental study, usually conducted in two species (usually mice, rats, or rabbits). Segment III is designed to evaluate peri- and postnatal toxicity in exposed dams and their offspring in one species (usually the rat). The highest dose used in all three segments generally is required to induce some form of minimal toxicity in the animal to ensure that an

### Table 2

A Rough Comparison of Physiological Maturation in Humans and Rats

<table>
<thead>
<tr>
<th>Time period</th>
<th>Human</th>
<th>Rat</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth to 1 month</td>
<td>Birth to 1 day</td>
<td>Transition from intrauterine environment to own capacity</td>
<td></td>
</tr>
<tr>
<td>Infancy</td>
<td>1 month</td>
<td>&gt;1 day</td>
<td>Lower metabolic capacity of many enzyme systems; period of rapid development</td>
</tr>
<tr>
<td>Childhood</td>
<td>6 months</td>
<td>&gt;5 days</td>
<td>Activity of most metabolic enzyme systems near, at, or greater than adult levels</td>
</tr>
<tr>
<td>Adolescence</td>
<td>12 years</td>
<td>&gt;8 weeks</td>
<td>Transition to adult metabolic activity; typical start time for subchronic and chronic studies in rats</td>
</tr>
<tr>
<td>Adult</td>
<td>18 years</td>
<td>&gt;6 months</td>
<td>Typical start time for clinical testing in humans and continued testing in rats</td>
</tr>
</tbody>
</table>

* Scheuplein et al. (2002) in part
adequate dose range is covered. FDA protocols for food additives include tests designed to detect effects on gonad function, estrous cycles, mating behavior, conception, parturition, pregnancy outcome, lactation, and postnatal growth and viability for up to three generations. Various agencies augment standard protocols by requiring behavioral assessments in Segment II and III studies.

The developmental toxicity study protocols used by the EPA and OECD are almost identical to the FDA Segment II teratology study. Those protocols are designed to evaluate the effects on soft tissues and skeletal development from exposures in utero during the periods of histogenesis and organogenesis.

The EPA (1991) published developmental neurotoxicity (DNT) testing guidelines (Supplement 10) for evaluating potential functional and morphological impacts of toxicants on the nervous system that may arise in offspring from exposure of the mother during pregnancy and lactation. Those guidelines have been recently updated by the EPA's Office of Prevention, Pesticides and Toxic Substances (U.S. EPA, 1998). The dosing period includes exposure in utero (starting gestation day 6) and during the postnatal period (through postnatal day 10). Animals are observed for 60 days for effects on developmental, behavioral, and neurological endpoints. Behavioral endpoints include learning and memory tests. The neurological observations include tests of motor activity and auditory startle; brain weights and neuropathological evaluations, including brain neuropathology, are recorded at the end of the study. Modifications of this protocol have been suggested, but whether this protocol or its modifications is acceptable as a useful adjunct to the traditional protocols needs further discussion within the scientific community. The fundamental problem with the new DNT testing requirements is that there are no published data demonstrating that such testing will reveal any neurological damage not also obtainable using a well-conducted two-generation reproductive study. The EPA does not recommend DNT testing for all substances, but only on a case-by-case basis depending on the toxicological information available for the chemical or class of chemical of interest. The EPA's Office of Toxic Substances has developed criteria for when DNT testing will be required (Francis et al., 1990; U.S. EPA, 1998).

Data Needs for “Safe” Dose Assessment

The specific number and types of toxicity tests used for safety assessment vary considerably across regulatory programs. The registration requirements of different countries for substances developed for specific biological activity, such as food use pesticides, are most stringent and can include many distinct mammalian toxicity tests. Similar requirements apply to pharmaceutical agents. Other assessment approaches, such as the OECD (1997) Screening Information Data Set (SIDs) process, follow a tiered approach, in which a set of toxicity studies is evaluated initially and, depending upon the results, the need for additional studies is determined. Similarly, the EPA's approach for evaluating "inert" ingredients in pesticide formulations consists of a set of tiered toxicity studies and guidelines for interpretation of results that lead to the triggering of more extensive toxicity studies.

Tiered approaches are typically applied to substances that are not specifically designed to be biologically active. Such chemicals differ in physical/chemical characteristics and in many other ways from pesticides and pharmaceuticals. Further, production processes and use patterns influence or limit intentional human exposures. An approach that begins with a limited core set of toxicity tests, such as the OECD (1997) SIDs, provides an efficient means of evaluating substances within the framework of a screening-level safety assessment and leads to setting additional testing priorities based on both toxicity concerns and exposure potential.

In the determination of safe doses from such studies, few investigators have discussed or agreed upon what comprises the necessary amount of appropriate data (see, for example, early work of Clegg, 1978). However, the EPA has used an uncertainty factor (based in part on earlier work of FDA) to estimate safe exposure levels in the absence of adequate data from multiple toxicity studies (Barnes and Dourson, 1988). This latter approach is now referred to as UFp (Dourson, 1994). The EPA considers this factor necessary because of the inability of any one study to adequately test different species or different life stages of the same species. The EPA has often found that the receipt of missing studies yields a different critical effect and a lower NOAEL. The EPA's use of UFp is based on its assumption that the critical effect can be discovered in a reasonably small selection of toxicity studies. In the consideration of setting safe exposure limits that protect children, evaluating the adequacy of and need for UFp has become important because of the concerns that incomplete toxicity testing will fail to identify effects relevant to children's health.

Initial attempts to understand how different toxicity studies identified the critical effect for safe exposure limits naturally focused on the frequency of different critical effects in the determination of such limits (see, for example, Fig. 3). Such evaluations included systemic toxicity in laboratory animals through acute, short-term, subchronic, and chronic studies; specialized testing, such as evaluations of developmental toxicity, reproductive toxicity, immunotoxicity, and neurotoxicity; and toxicokinetic and toxicodynamic evaluations. If available, all of these studies are used to characterize a chemical's spectrum of potential human toxicity by identifying target organs and the dose ranges
associated with adverse effects in laboratory animals of different life stages.3

Unfortunately, a problem with these initial evaluations is quickly evident. Quite simply, the databases for many chemicals lack a sufficient number of studies that evaluate different endpoints and life stages. Thus, the results in Fig. 3, which illustrate that ~9% of all EPA RfDs are based on reproductive or developmental toxicity studies, do not give much assurance that this percentage represents an accurate estimate of the number of times these effects might serve as the basis of exposure limits if complete chemical-specific databases were more widely available.

The EPA conducted further work on the impact of missing data in developing RfDs, including data for different life stages, and this research directly relates to evaluations of the usefulness of UFp (Dourson et al., 1992). For example, data for 69 pesticides were analyzed and frequency histograms of NOAEL ratios were developed for chronic dog, mouse, and rat toxicity studies and for rat reproductive and developmental toxicity studies (see Fig. 4). These pesticides were selected because of the availability of many different toxicity studies on both adult and young animals. On average, chronic rat and dog studies, generally conducted on young adult to older animals, yielded similar NOAELs. Reproductive and developmental toxicity studies, conducted on both adult and young animals, were less likely to produce the lowest NOAELs when compared to the chronic rat and dog studies. Chronic mouse studies, generally conducted on young adult to adult animals, were least likely to yield the lowest NOAEL when compared to the chronic rat and dog studies and thus only occasionally resulted in the determination of a critical effect. The authors concluded that several bioassays are needed in order to develop a high confidence estimate for an RfD and, if one or more bioassays is missing (which is often the case when developing RfDs and other safe doses), then a factor such as UFp could be supported quantitatively. Specifically, when chronic rat and dog studies are available but rat reproductive and rat developmental toxicity studies are missing, a UFp of 3 applied to the lower of the chronic rat or dog NOAEL accounts for $\sim$92% of the possible occurrences of lower NOAELs being identified by the missing bioassays that include younger animals. A UFp of 10 accounts for 98% of such occurrences.4 Therefore, the routine use of UFp

3 In vitro data can be used to elucidate potential mechanisms of biological activity, to evaluate the relevance to humans of the endpoint observed in laboratory animals, to improve extrapolation from laboratory animals to humans, and to characterize intrahuman variability. Assessment of laboratory animal data should include an evaluation of the reliability of the experimental design and toxicological interpretation of the results. Moreover, once a critical effect and likely mode of action have been identified, results from the various studies should be examined collectively to determine whether a causal relationship is likely to exist between a chemical exposure and the hypothetical human effect. Species-specific differences in sensitivity to a chemical due to differing metabolism, physiology, or anatomy, also should be considered.

4 The specific comparison made is found in Dourson et al. (1992), Table 6, line 18. The values of 0.08 at $10^{0.5}$ and 0.02 at $10^{1.0}$ are the probabilities that either the rat reproductive or rat developmental toxicity study NOAELs are lower than the corresponding NOAELs for either the chronic dog or the rat bioassays. Thus, the chronic bioassay NOAELs, when divided by an uncertainty factor of 3 ($10^{0.5}$) or 10 ($10^{1.0}$), protect against either 92 or 98% of the potentially lower NOAELs that could be identified by bioassays that include younger animals, respectively.
by the EPA to compensate for the lack of certain bioassays already addresses, in large part, the uncertainty associated with the absence of specific studies, including studies that test younger animals.

Baird et al. (1996) presented two approaches for estimating the quantitative value of UF_D using a subset of studies on pesticides identified by Dourson et al. (1992), discussed above. One method, based on regression analysis, provided a point estimate of UF_D. The other method, based on nonparametric analysis, intended to provide a distributional estimate of UF_D. In both cases, the choice of UF_D depended on the definition of a complete database (see the EPA's described below), the number of missing bioassays, and the specific bioassay missing.

Based in part on the analysis of Dourson et al. (1992) of pesticides and published criteria for causal significance of Hill (1965), the EPA routinely uses UF_D to determine RfDs in cases where certain bioassays are missing, including when studies are missing that test younger animals. This use allows the EPA to confidently develop RfDs for many compounds without the full complement of toxicity tests. The EPA generally considers a "complete" database—that is, complete for the purpose of estimating RfDs or RfCs for noncancer health effects with "high" confidence and no use of the database uncertainty factor—to comprise the following:

- two adequate mammalian chronic toxicity studies by the appropriate route of exposure in different species;
- one adequate mammalian multigeneration reproductive toxicity study by the appropriate route of exposure;
- two adequate mammalian developmental toxicity studies by the appropriate route of exposure in different species.

This series of tests is considered complete because most of the animals' life stages will have been investigated (see Fig. 1). The judgment of a complete database is somewhat chemical-specific, however; the observation of certain types of toxicity (e.g., neurotoxicity) in short-term tests may suggest the need for specialized tests not included in the general definition of a complete database.

For example, Makris et al. (1998) investigated the usefulness of developmental neurotoxicity (DN) tests

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5 Generally, the presence of a complete database indicates that the acquisition of additional toxicity data is unlikely to result in a change to the RfD or RfC. Scientists at the EPA typically consider such RfDs and RfCs to be "high confidence," reflecting the likely stability of the value to additional data. The EPA considers a single, well-conducted, subchronic mammalian bioassay by the appropriate route as a minimum database for estimating a RfD or RfC. However, for such a limited database, the likelihood that additional toxicity data may change the value of the RfD or RfC is higher, and the associated confidence in them is lower. Due to the conservatism inherent in the uncertainty factor approach, the acquisition of additional data often results in higher RfDs and RfCs (i.e., results in the conclusion that higher exposures are safe). For more details please see U.S. EPA (1994) or Dourson (1994). Examples of confidence statements for RfDs and RfCs can be found in the EPA's online IRIS database (www.epa.gov/iris).

6 As determined by professional judgment. Typically, studies should have been adequately conducted and published in refereed journals or be unpublished reports that adhered to Good Laboratory Practice guidelines and have undergone final QA/QC (U.S. EPA, 1994). The EPA and others have published guidelines in this area. For example, see U.S. EPA (1998) and FDA (1993).
as part of this database by comparing DN NOAELs to NOAELs derived from other types of toxicity tests. They found that for nine of the pesticides investigated, eight DN NOAELs were lower than developmental toxicity NOAELs, six were lower than reproductive toxicity NOAELs, and six were lower than or approximately equal to neurotoxicity NOAELs. However, DN NOAELs were between 13- and 93-fold higher than the NOAELs used as the basis of the lifetime RfDs for seven of these same pesticides. For the remaining two pesticides, DN NOAELs were 70 and 90% of the chronic NOAELs. The mean difference between DN and chronic NOAELs was 25-fold, suggesting that DN NOAELs are generally much less sensitive that chronic NOAELs.

Moreover, a peer review of Makris et al. (1998) concluded that either maternal toxicity or developmental toxicity generally occurs at comparable or lower dose levels than developmental neurotoxicity (SAP, 1999). The peer review showed that for 10 of the 12 substances evaluated, either the maternal toxicity NOAEL or the developmental toxicity NOAEL was the same as or less than the DN NOAEL. In only 1 case was the DN NOAEL less than either the maternal toxicity NOAEL or the developmental toxicity NOAEL, and in this case the effect reported for the DN NOAEL was questioned by the peer review. With respect to the applicability and sensitivity of the DN study, the majority of the peer review panel strongly indicated that the DN study was not more sensitive than either the developmental study or the reproductive study.

Thus, while the DN study may (or may not) be more sensitive in some cases than other specialized studies, its overall contribution to the determination of a lifetime RfD is likely to be minimal, because it is not as sensitive as chronic bioassays. Its use in the development of higher acute or other less than lifetime RfDs is perhaps more likely, because in these situations, lifetime studies are seldom used.

Overall, an uncertainty factor of 3 or 10 commonly used by the EPA for varying degrees of database incompleteness seems appropriate and more than adequate when information suggests that developmental, reproductive, or developmental neurotoxicity may be the critical effect in the absence of specific information. This conclusion is based on a fair number of pesticides, but could be enhanced with reviews on other types of chemicals.

VARIABILITY, UNCERTAINTY, AND DIFFERENTIAL SENSITIVITY

Individual susceptibility depends on both toxicokinetic and toxicodynamic mechanisms, and these mechanisms may be classified into three types: factors that increase the concentration of active substance at the critical target tissue; factors that augment the reaction of the active substance with the target tissue; and factors that promote the sequence of events between the initial reaction and final adverse effect (Grandjean, 1992).

An uncertainty factor of 10, commonly referred to as UFH, is generally used to account for the variability in response between the population mean and highly sensitive subjects within the human population (IPCS, 1994). The value of 10 is used for UFH as a default; that is, 10 is used unless there are data indicating that a different value is more appropriate. Use of UFH assumes that there is variability in response to chemical toxicity from one human to the next and that this variability may not have been detected in the epidemiology study, usually due to factors such as small sample size. Use of this factor may also assume that groups of humans exist, such as children, the elderly, or those with genetic polymorphisms that predispose them to unique sensitivity when compared with the average population (e.g., a bimodal distribution of sensitivity; see below for further discussion). A recent review of this default factor indicates that it is relatively robust, with greater than 99% of the population, including sensitive subgroups, being protected (Burin and Saunders, 1999).

8 For the purposes of this text, we define toxicokinetics as the chemical's absorption, metabolism (excluding target tissue metabolism), distribution, and elimination. Others have defined toxicokinetics as above but without the exclusion of target tissue metabolism. We use our definition because of expediency. Quite simply, the majority of toxicokinetic data we analyze are measurements of AUC or clearance (see comparisons shown in Table 4), and these parameters appropriately represent the variability in kinetics that exclude target tissue metabolism. Furthermore, most of the studies looking at kinetic variability are looking at the parent compound. For pharmaceuticals for which the active agent is a metabolite, the active metabolite is usually (although not always) generated in a different tissue than the target tissue, and our definition of kinetics will properly note expected variability. In contrast, toxicity for environmental chemicals may more often result from generation of the active metabolite in the target tissue. Variability in this target tissue metabolism would not be included in our definition of toxicokinetics, and therefore our conclusions of toxicokinetic variability will be lacking. These are complex issues and will require additional research and discussion to fully sort out.

9 While UFH seeks to provide protection for sensitive members of the population, IPCS (1994) specifically states that "idiopathic hyperresponsiveness (excessive reaction following exposure to a given dose of a substance compared with the large majority of those exposed to the same dose) in a few individuals would not be the basis for the derivation of the TI (Tolerable Intake, which is synonymous with an RfD). . . ." This caveat is used by other groups as well.

7 In the case of specific information on these endpoints, the choice of NOAEL or LOAEL of the critical effect becomes more definitive. For example, when such endpoints are the critical effect, then the lifetime RfD is based on their NOAEL, even though the study is of shorter duration.
DIFFERENTIAL RISK BETWEEN CHILDREN AND ADULTS

Interpreting $UF_H$

Significant misunderstanding about what $UF_H$ represents is apparent from the literature. The use of $UF_H$ applied to the NOAEL or BMD is not expected to reflect the complete distribution of human sensitivities, as some investigators in this area have suggested, or even the population mean to the highly sensitive subjects within the human population as mentioned above. Rather, applying $UF_H$ to the projected NOAEL or BMD reflects the range of sensitivities expected between the lower range of a normal distribution in the overall population and the sensitive subgroup (Dourson et al., 1996).10

$UF_H$ is commonly applied to the NOAEL or BMD estimated for humans. Human NOAELs or BMDs are usually projected from those observed in laboratory animals by dividing by another uncertainty factor, $UF_A$ (not discussed here), which is meant to account for differences in sensitivity between species (see Fig. 5a). This projected NOAEL or BMD for humans is expected to reflect the rate of response in the lower range of a normal distribution of human responses, because this is what the NOAEL or BMD reflects in the animal study (see Fig. 5b). In some cases the NOAEL and BMD can be measured or estimated from human studies. If so, some assurance is needed that the NOAEL and BMD are not derived from a subpopulation of resistant individuals. In that case, the NOAEL and BMD might not reflect the rate of response in the lower range of a normal distribution of human responses, by definition.

Figure 6a shows a trimodal distribution composed of sensitive, average, and resistant humans.11 When interpreted properly and starting from a NOAEL or BMD of the average group of humans, $UF_H$ accounts for overall variability in the human population of much greater than 10-fold, perhaps between 100- and 1000-fold or more (see also Fig. 6b; variability spans approximately 3 orders of magnitude). Such appropriate interpretation also allows modification of $UF_H$ when NOAELs are available for a known sensitive or resistant human subgroup or if human toxicokinetics or toxicodynamics are known with some certainty. In such cases, $UF_H$ should be adjusted (either increased or decreased) or replaced accordingly.

As an example of how one agency approaches the use of $UF_H$, Table 3 shows a comparison of 24 RfDs based on human data as found on IRIS (U.S. EPA, 2001). Of these 24 values, 4 of them are based on a critical effect found in children, who are known to be the sensitive subgroup (fluorine, methyl mercury, nitrate, and nitrite). Five additional values are based on large population studies, which may have included children, but are also judged to include at least some sensitive individuals (arsenic, benzoic acid, cadmium, manganese, and selenium). In all but 2 of these 9 cases, $UF_H$ was reduced from its 10-fold default value to either 3 (arsenic and selenium) or even 1 (benzoic acid, fluorine, manganese, nitrate, and nitrite). Reductions of $UF_H$ are judged appropriate when sufficient data are available to suggest that there are unlikely to be any sensitive subgroups or where the RfD is based on a NOAEL or BMD from a sensitive human subgroup.

Adequacy of $UF_H$ for Adults

A number of scientists have investigated whether $UF_H$ accounts quantitatively for the variability to chemical toxicity between the overall human population and its potentially more sensitive groups. Such studies are most useful in addressing the adequacy of $UF_H$. Other investigators have generated data that are less useful for evaluating the adequacy of $UF_H$, because the research was done for some other purpose. Both types

10 Price et al. (1999) have written a very nice text that explains this issue using both a finite sample size model (which we represent in Fig. 5) and a sensitive population model (which we represent in Fig. 6).

11 Note here that the NOAEL or BMD appears to be less than the 10% mark shown more clearly in Fig. 5b. However, the NOAEL in Fig. 6a is the same as that in Fig. 5b. In the case of Fig. 6a, two populations must be added to obtain the 10% response, that of the sensitive and average humans.
of studies are summarized in Table 4 and described below.

Dourson and Stara (1983) analyzed acute toxicity data for 490 chemicals from Weil (1972), finding that for about 92% of the chemicals, a 10-fold UFH would yield a 3-probit reduction from a median response (i.e., 99.9% of the population would be protected). Brown (2001) compared ED50’s or LD50’s for critically ill or injured patients or laboratory animals and found that a UFH of 10 applied to the normal population’s ED50 or LD50 protected to the lower 5th percentile of the compromised population 97% of the time. Hattis et al. (1999a,b) projected the incidence of effect that would be expected (b) Cumulative response as a function of dose for humans of different sensitivities. Hypothetical data for humans are the same as in (b).

(b) Cumulative response as a function of dose for humans of different sensitivities. Data are the same as in (a).

of studies, the author concluded that the 10-fold factor appeared to provide protection for up to about 80–95% of the public. Again, however, that conclusion was based on the supposition that UFH is meant to account for the total range of human variability. Actual use of UFH suggests that it protects more of the population than this author concluded (see section on interpreting UFH).

Some of the studies evaluating intrahuman sensitivity looked separately at the 3.16-fold toxicokinetic and toxicodynamic components of UFH.12 Renwick and Lazarus (1998) investigated a database comprising 60 compounds with metabolism and clearance data and 49 compounds with effects data. The authors found that a kinetic uncertainty factor of 3.16 failed to protect a mean of 0.0685 and 0.8564% (precision as reported by the authors) of the population, respectively, when a normal or lognormal distribution for the population variability was assumed. A dynamic uncertainty factor of 3.16 failed to protect mean values of 0.2930 and 1.8896% of the population, respectively. If the kinetic and dynamic aspects of UFH are assumed to be independent, combining both factors to yield the default value of 10 (i.e., 3.16 × 3.16) protected 99.9998% and 99.9998% of the population, respectively. However, specific subpopulation comparisons with preterm infants and children or different ethnic groups occasionally lead to greater variation when compared to the standard adult population. Such subpopulation comparisons indicate the

12 The value of 3.16 comes from equal subdivision of the default 10-fold UFH into its kinetics and dynamics components. If independent action is assumed, then 3.16 × 3.16 = 10. This factor is also often shown as 3.2 or 3, because of concerns about portraying uncertainty factors as being overly precise. With any of these designations, however, the intent is to show one-half log10 values. This representation often leads to the somewhat confusing nomenclature found on the EPAs IRIS and elsewhere suggesting that 3 × 3 = 10.
### TABLE 3
Summary of U.S. EPA's RfDs on IRIS as of May 2000 Based on Human Data

<table>
<thead>
<tr>
<th>Chemical name (as on the EPA's IRIS)</th>
<th>Specstype of study</th>
<th>NOAEL, LOAEL, or BMD</th>
<th>Critical effect(s)</th>
<th>Uncertainty factor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RfD</th>
<th>RfD confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldicarb</td>
<td>Human experimental gavage</td>
<td>0.01</td>
<td>Clinical signs of blood or plasma cholinesterase inhibition</td>
<td>Total 10 1 1 1 1 1 1</td>
<td>1 E-3 Medium</td>
<td></td>
</tr>
<tr>
<td>Arsenic, inorganic</td>
<td>Human epidemiology drinking water</td>
<td>0.0008</td>
<td>Skin lesions and possible vascular complications</td>
<td>3 3 1 1 1 1 1</td>
<td>3 E-4 Medium</td>
<td></td>
</tr>
<tr>
<td>Barium</td>
<td>Human experimental, epidemiological drinking water</td>
<td>0.21</td>
<td>Increased blood pressure</td>
<td>3 3 1 1</td>
<td>7 E-2 Medium</td>
<td></td>
</tr>
<tr>
<td>Baygon</td>
<td>Human experimental single dose</td>
<td>0.36 (L)</td>
<td>Mild cholinergic symptoms, RBC cholinesterase inhibition</td>
<td>100 10 1 10 1 1 1</td>
<td>4 E-3 Medium</td>
<td></td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>Human anecdotal dietary exposure</td>
<td>4.4</td>
<td>No adverse effects observed</td>
<td>1 1 1</td>
<td>4 E-4 Medium</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>Human chronic exposures from a variety of studies</td>
<td>0.005</td>
<td>Significant proteinuria</td>
<td>10 1 1</td>
<td>5 E-4 High</td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Human experimental capsule</td>
<td>0.03</td>
<td>Plasma cholinesterase inhibition</td>
<td>10 1 1 1</td>
<td>3 E-3 Medium</td>
<td></td>
</tr>
<tr>
<td>4,6-Dinitro-o-cyclohexyl phenol</td>
<td>Human anecdotal clinical therapy</td>
<td>2.0 (L)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Cataract formation</td>
<td>1000 10 1 10 1 1 1</td>
<td>2 E-3 Low</td>
<td></td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>Human anecdotal clinical therapy</td>
<td>2.0 (L)</td>
<td>Cataract formation</td>
<td>1000 10 1 10 1 1 1</td>
<td>2 E-3 Low</td>
<td></td>
</tr>
<tr>
<td>Etephon</td>
<td>Human experimental oral exposure</td>
<td>0.5 (L)</td>
<td>Plasma cholinesterase inhibition</td>
<td>10 1 1 1 1</td>
<td>5 E-3 Low</td>
<td></td>
</tr>
<tr>
<td>Ethion</td>
<td>Human experimental short term</td>
<td>0.06&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Plasma cholinesterase inhibition</td>
<td>10 1 1 1</td>
<td>5 E-4 Medium</td>
<td></td>
</tr>
<tr>
<td>Fluorine (soluble fluoride)</td>
<td>Human epidemiology</td>
<td>0.06</td>
<td>Objectionable dental fluorosis in children</td>
<td>1 1 1 1</td>
<td>6 E-2 High</td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>Human experimental feeding</td>
<td>0.23</td>
<td>Erythrocyte cholinesterase depression</td>
<td>10 1 1 1</td>
<td>2 E-2 Medium</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>Human data of several types</td>
<td>0.14</td>
<td>No LOAEL given, CNS effects appear to occur at higher doses</td>
<td>1 1 1</td>
<td>1.4 E-3 Medium</td>
<td></td>
</tr>
<tr>
<td>Methylmercury</td>
<td>Human epidemiological poisoning</td>
<td>0.000657 to 0.001472 (B)</td>
<td>Infant developmental neurological abnormalities</td>
<td>10 1 1 1 1</td>
<td>1 E-4 High</td>
<td></td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Human epidemiological dietary surveys</td>
<td>0.14 (L)</td>
<td>Increased uric acid</td>
<td>30 3 1 10 1 1</td>
<td>5 E-3 Medium</td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>Human epidemiology surveys</td>
<td>1.8</td>
<td>Early clinical signs of methemoglobinemia in children &lt;10%</td>
<td>1 1 1</td>
<td>1.6 E+0 High</td>
<td></td>
</tr>
<tr>
<td>Nitrite</td>
<td>Human epidemiology surveys</td>
<td>1.0&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Early clinical signs of methemoglobinemia in children &lt;10%</td>
<td>1 1 1</td>
<td>1 E-1 High</td>
<td></td>
</tr>
<tr>
<td>Pirimiphosmethyl</td>
<td>Human 56-day experimental feeding</td>
<td>0.25</td>
<td>Transient plasma cholinesterase inhibition</td>
<td>25 10 1 2 1</td>
<td>1 E-2 High</td>
<td></td>
</tr>
<tr>
<td>Selenium and compounds</td>
<td>Human food and soil epidemiology</td>
<td>0.015</td>
<td>Clinical senility</td>
<td>3 3 1 1</td>
<td>5 E-3 High</td>
<td></td>
</tr>
<tr>
<td>Silver</td>
<td>Human anecdotal studies</td>
<td>0.014</td>
<td>Argyria</td>
<td>3 3 1 1 1</td>
<td>5E-3 Low</td>
<td></td>
</tr>
<tr>
<td>1,1,2-Trichloro-1,2,2-trifluoroethane</td>
<td>Human occupational exposure</td>
<td>273</td>
<td>Psychomotor impairment</td>
<td>10 1 1</td>
<td>3E+1 Low</td>
<td></td>
</tr>
<tr>
<td>Warfarin</td>
<td>Human experimental diet supplement</td>
<td>0.029</td>
<td>Increased prothrombin time</td>
<td>100 10 1 1 1</td>
<td>3E-4 Low</td>
<td></td>
</tr>
<tr>
<td>Zinc and compounds</td>
<td>Human experimental</td>
<td>8.97 (L)</td>
<td>Decrease in erythrocyte superoxide diamutase concentration in adults</td>
<td>100 10 1 1</td>
<td>3E-1 Medium</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> All values are in mg/kg-day and are NOAELs unless otherwise stated: (L), LOAEL; (B), benchmark dose (BMD).

<sup>b</sup> Uncertainty factors are H, average human to sensitive human; A, animal to human; L, LOAEL to NOAEL; S, subchronic exposure to chronic; D, database insufficiency; MF, modifying factor to account for uncertainties not covered by the traditional factors.

<sup>c</sup> Based on analogy to 2,4-dinitrophenol.

<sup>d</sup> The EPA's IRIS lists this value as a NOEL, but also adds an uncertainty factor for LOAEL to NOAEL extrapolation because of the proximity of this NOEL to brain cholinesterase inhibition in dogs at 0.71 mg/kg-day.

<sup>e</sup> Based on the toxicity of nitrate with an adjustment to this dose with a 10-fold MF.
<table>
<thead>
<tr>
<th>Investigators</th>
<th>Percentage of chemicals or population protected by UF₉ of 10 or subfactor of 3.16</th>
<th>UF₉ for 99% protection</th>
<th>Starting point</th>
<th>Data set and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dourson and Stas, 1983</td>
<td>For 92% of the chemicals tested, 99.9% of the population protected</td>
<td>6</td>
<td>Animal LD₅₀ analysis of log probit slopes</td>
<td>Assumes that UF₉ of 10 covers from the average to the sensitive human response.</td>
</tr>
<tr>
<td>Brown, 2001</td>
<td>97% protection of the 5th percentile of a compromised population</td>
<td>Not estimated</td>
<td>KLD₅₀ or LD₅₀ of the normal or compromised population compared</td>
<td>Interhuman variability in critically ill or injured subjects.</td>
</tr>
<tr>
<td>Hattis et al., 1999a, b</td>
<td>99.99 to 99.9% of the population</td>
<td>Not estimated</td>
<td>5% response level in a normal population</td>
<td>Based on assumption that population sensitivity is logarithmic and includes parameters related to health risks in adults and some children.</td>
</tr>
<tr>
<td>Brock, 1991</td>
<td>99.97% of the population</td>
<td>Not estimated</td>
<td>Human variation in cholinesterase inhibition among and within individuals</td>
<td>Comparison given here is for a specific dynamic parameter. The proper comparison of these ratios is to the dynamic factor of 3.16 for UF₉.</td>
</tr>
<tr>
<td>Hattis et al., 1987</td>
<td>97% of the population</td>
<td>15</td>
<td>Human kinetic parameters</td>
<td>Based on toxicokinetic parameters in healthy adults. Assumed that UF₉ of 10 covered the whole range of the human population. This early work did not separately evaluate the kinetic subpart of 3.16 for UF₉.</td>
</tr>
<tr>
<td>Calabrese, 1985</td>
<td>80 to 95% of the population</td>
<td>Not estimated</td>
<td>None given</td>
<td>Comparisons are for both effects and specific kinetic or dynamic parameters. Thus, the proper comparison of these ratios might be to either the full 10-fold UF₉ or to the 3.16-fold component, which makes the interpretation of this study difficult. Furthermore, the author assumed that UF₉ of 10 covered the whole range of the human population (see text for a discussion of this error).</td>
</tr>
<tr>
<td>Renwick and Lazarus, 1986</td>
<td>99.998 to 99.9838% of the population</td>
<td>Not estimated</td>
<td>Analysis of variability in kinetic and dynamic parameters</td>
<td>Assumes that the kinetic and dynamic aspects of UF₉ are independent; children do not appear, in general, to represent a sensitive subgroup from the kinetic aspects; genetic polymorphisms or ethnic differences may decrease the overall proportion of the population protected by a UF₉ of 10.</td>
</tr>
<tr>
<td>Renwick et al., 2001</td>
<td>99.9% of the population</td>
<td>2.6</td>
<td>Human variability in kinetic parameters associated with CYP1A2 metabolism</td>
<td>Analysis based on 3.16 kinetic part of UF₉ in healthy adult populations.</td>
</tr>
<tr>
<td>Silverman et al., 1999</td>
<td>For 80% of chemical-specific kinetic parameters, 96 to 99.999% of the population protected</td>
<td>Not estimated</td>
<td>Human kinetic default uncertainty factor of 3.2</td>
<td>Human kinetic variability (i.e., AUC and Cmax in response to 6 drugs; some information on children available but insufficient for separate conclusions.</td>
</tr>
<tr>
<td>Glaubiger et al., 1982</td>
<td>100% of the chemicals</td>
<td>Not estimated</td>
<td>Human MTDs of the anticancer drugs in children and adults</td>
<td>Study was not designed to test the adequacy of UF₉; mean values do not allow distributions of sensitivities between groups.</td>
</tr>
<tr>
<td>NAS, 1985; Charnley and Putzrath, 2001</td>
<td>Not estimated</td>
<td>Not estimated</td>
<td>Animal comparisons of chemically induced carcinogenesis based on age</td>
<td>Younger animals are less susceptible than adults for 47% of the time, equally sensitive for 13%, and more sensitive for 40%.</td>
</tr>
<tr>
<td>Study</td>
<td>Population</td>
<td>Toxicokinetic Factors</td>
<td>Data Source</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>----------------</td>
<td>---------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Sheehan and Gaylor, 1990</td>
<td>86% of the chemicals</td>
<td>Not estimated</td>
<td>Animal LD&lt;sub&gt;50&lt;/sub&gt; ratios of adult to younger</td>
<td></td>
</tr>
<tr>
<td>Calabrese, 2001</td>
<td>86% of the chemicals</td>
<td>Not estimated</td>
<td>Data are from 238 chemicals, but information given in an abstract only.</td>
<td></td>
</tr>
<tr>
<td>Rane, 1992</td>
<td>67% of the chemicals</td>
<td>Not estimated</td>
<td>Data are from 313 chemicals in 25 chemical classes.</td>
<td></td>
</tr>
<tr>
<td>Renwick, 1998</td>
<td>91% of the chemicals</td>
<td>Not estimated</td>
<td>Analysis based on 3.16 kinetic part of UF&lt;sub&gt;H&lt;/sub&gt;.</td>
<td></td>
</tr>
<tr>
<td>Naumann, 2001</td>
<td>All values are for % of the population</td>
<td>Not estimated, NE, not estimated</td>
<td>Analysis based on 3.16 kinetic part of UF&lt;sub&gt;H&lt;/sub&gt;.</td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
<td>Dynamics: 100</td>
<td>Human kinetic and dynamic values compared among different subpopulations including aged, children, normal, and those with advanced disease</td>
<td>Data are from five classes of drugs. Subfactors for dynamics and kinetics were not combined as in Renwick and Lazarus (1998) shown above, and thus the protectiveness of the overall default value of 10 for UF&lt;sub&gt;H&lt;/sub&gt; is not evaluated by these investigators.</td>
<td></td>
</tr>
<tr>
<td>Angiotensin inhibitors</td>
<td>Dynamics: 100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinetics: 96</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsteroidal antiinflammatory</td>
<td>Dynamics: NE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinetics: 60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol-lowering drugs</td>
<td>Dynamics: NE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinetics: 86</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Dynamics: NE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinetics: 90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skowronski and Abdel-Rahman, 2001</td>
<td>100% of 6 drugs studied</td>
<td>Not estimated</td>
<td>Toxicokinetic factors compared among children, adults, and elderly</td>
<td></td>
</tr>
<tr>
<td>Ginsberg et al., 2002</td>
<td>Not estimated, but would vary among different drugs studied and age of the child.</td>
<td>Not estimated</td>
<td>Data were analyzed the composite factor of 10 using comparative kinetic data and the IPCS default value for toxicodynamic variability. All estimated composite values were less than 10. Study was specifically designed to compare adult and child toxicokinetic parameters. Several different stages of childhood were studied. Premature and full-term neonates and newborns have a statistically significant higher average half-life and correspondingly lower average clearance when compared with adult values. Older children have statistically significant higher average clearances. Age-specific adjustment factors were recommended when sufficient data on a chemical are available.</td>
<td></td>
</tr>
</tbody>
</table>
need for caution in stating the protectiveness of default uncertainty factors and suggest the need for additional research on polymorphisms as they relate to population variability in toxic response. Renwick et al. (2001) also studied human variability in kinetic parameters associated with CYP1A2 metabolism, finding differences within healthy populations of 1.8 or 2.4 for the 95th or 99th percentiles, respectively.

Silverman et al. (1999) looked at intrahuman variability in pharmacokinetic parameters from published pharmaceutical clinical trial data for six compounds. Specifically, the authors examined the area under the chemical concentration-time curve (AUC) and the peak plasma concentration (Cmax). The purpose of their work was to investigate the use of specific data describing intrahuman variability in lieu of the usual default UFH value of 10. Review of the data indicated that the default value of 3.2 for the kinetic component of UFH was more than sufficient in 12 of 15 cases. For those cases, a relatively small percentage of the population (0.001 to 3.8%) would be left unprotected if kinetic data were substituted for the default kinetic value and combined with the default value of 3.2 for the dynamic component of UFH. In the remaining 3 instances, a composite factor greater than the usual default UFH value of 10 would be required to provide adequate coverage (defined by the authors as 95% of the sensitive population).

Adequacy of UFH for Children

Although the studies described above indicate that a 10-fold UFH generally protects individuals with greater-than-average sensitivities, recent concern has focused on the adequacy of UFH for protecting children in particular. This concern has prompted evaluations of the differences in susceptibility to chemical toxicity between younger animals and older animals and of differences in clinical sensitivities to pharmaceutical agents in children compared to adults for a variety of agents. The studies discussed here represent the few that permit quantitative evaluation of age-related differences in sensitivity for the purpose of evaluating the adequacy of UFH for children. These studies are also summarized in Table 4 and described below.

Glaubiger et al. (1982) compared maximum tolerated doses (MTDs)13 for 17 anticancer drugs in children and adults. Ratios of the child MTD to the adult MTD, when measured as milligrams per square meter of surface area or milligrams per kilogram per day, varied from 0.83 to greater than 2.2. Three ratios were less than 1; 1 ratio equaled 1; and 13 ratios were greater than 1. Ratios greater than 1 indicated that the child was less sensitive than the adult. The mean ratio was at least 1.3. However, if all of the ratios were based on milligram per kilogram per day doses, which is the dose measurement on which uncertainty factors are based, the ratios varied from about 1.3 to over 4.1, with a mean ratio of 2.3. As before, ratios greater than 1 indicate that children are less sensitive than adults to the toxicity of these chemicals. Because these ratios are for an effect, not for a specific kinetic or dynamic parameter as discussed elsewhere in this text, the proper comparison of the ratios is to the full 10-fold UFH. Thus, for each of the 17 chemicals evaluated, children are less sensitive than adults on a milligram per kilogram per day basis, although the overall difference in sensitivity between children and adults is quite small as measured by mean ratios.

The National Academy of Sciences report Pesticides in the Diets of Infants and Children (NAS, 1983) included a table summarizing the results of studies that had been performed through 1983 in which the effects of age on chemically induced carcinogenesis in rodents had been evaluated. Charnley and Putzrath (2001) updated those results to include studies performed since 1983. The data indicate that there are a similar number of studies demonstrating that younger animals are less susceptible than adults (47%) to chemically induced carcinogenesis as there are demonstrating that they are more susceptible (40%) under the conditions of the bioassays. A number of studies showed that age played no role at all in susceptibility (13%). The extent of the age-related differences was not evaluated quantitatively because virtually all of the studies were performed using only one high dose level, so the underlying dose–response relationships are unknown. The NAS report concluded that those results clearly demonstrate that age may be an important factor in susceptibility to chemically induced carcinogenesis, but they do not support the conclusion that younger animals are always more susceptible than older animals. The NAS went on to further conclude that UFH of 10-fold provides adequate protection of infants and children, based on current knowledge (Bruckner, 2000).

Sheehan and Gaylor (1990) compared the LD50 ratios of adult to newborn mammals for 238 chemicals as a measure of intraspecies variability. The median ratio was 2.6 (adult to newborn). In contrast to Glaubiger et al. (1982) above, ratios greater than 1 indicate that the child is more sensitive than the adult. The percentage of LD50 ratios less than 10 was ~96%. Because the ratios given here are for an effect and not a specific kinetic or dynamic parameter, the proper comparison is to the full 10-fold UFH. Thus, for the chemicals evaluated, young animals on average were more sensitive than adults to acute lethality, and the 10-fold UFH would

13 It should be noted here and with previous and subsequent discussion of LD50's that such high-dose studies invariably use gavage or bolus dosing, resulting in peak loads that can saturate detoxification mechanisms in newborns. Similar doses given at rates that simulate environmental exposure might be detoxified more efficiently, and the values of adult to younger human or animal MTDs or LD50's might be more similar.
adjust the adult animal LD50 to that of the younger animals for 86% of the chemicals tested.

Calabrese (2001) showed a similar analysis of age comparisons for LD50 determinations in laboratory animals for 313 chemicals. Ninety-seven adult to young LD50 ratios (31%) were between 0.5 and less than 2.0, indicating that adults and young animals were equally sensitive. Adults displayed greater sensitivity in 46 cases (14%), exceeding 10-fold greater sensitivity than younger animals in 4 cases (1%). Younger animals displayed greater sensitivity in 170 cases (54%), exceeding 10-fold greater sensitivity than older animals in 43 cases (14%). Again, young animals were found on average to be more sensitive than adults to acute lethality and the full 10-fold UFH would adjust the adult animal LD50 to that of the younger animal for 86% of the chemicals tested.

Rane (1992) compared the elimination half-lives in newborn and adult humans for 14 drugs of known hepatic clearance. He illustrated that newborns were poorer at clearance when compared with adults for the majority of chemicals studied (i.e., 10 of 14) and thus presumably would be more sensitive to their toxicity; that is, newborns were exposed to a larger internal dose of those chemicals than adults. The ratios of newborn to adult kinetic parameters varied from 0.60 to 17;14 ratios greater than 1.0 suggest that the child was more sensitive than the adult. The arithmetic average was 3.5. Because the comparison given by Rane (1992) is for a specific kinetic parameter and not for an effect, as discussed in each of the three studies on LD50 or MTD, the proper comparison for these ratios is to the 3.16 kinetic component of UFH. On that basis, infants and children on average are less sensitive than adults to chemical toxicity (i.e., the average ratio is greater than 1.0), but when they are not, the 3.16 kinetic component of UFH adjusts the adult human kinetic parameter to that of the infant or child for 91% of the chemicals tested (i.e.,

children have more rapid clearance rates when compared with adults for the majority of chemicals studied (16 of 22) and concluded that infants and children are, therefore, exposed to lower doses of those chemicals than adults. The arithmetic average ratio of infant or child to adult kinetic parameters was 1.8 as determined by us using the data provided by Renwick (1998); ratios greater than 1.0 suggest that the infant or child is less sensitive than the adult because clearances (generally) are being compared. [In contrast, Rane (1992) compared half-lives.] Moreover, when infants or children eliminated chemicals more slowly than adults, that difference was no greater than 5-fold based on the mean ratios of infant or child to adult kinetic parameters (see Fig. 7). Variations within the adult and infant or child subgroups, when given, appear to be similar. Renwick concluded that the higher clearance of many xenobiotics by children compared with adults may compensate, at least in part, for increased organ sensitivity during development and that an increased uncertainty factor for postsuckling infants and children is not required. As above, because the comparison given by Renwick is for a specific kinetic parameter and not for an effect, the proper comparison of these ratios is to the 3.16 kinetic component of UFH. On that basis, infants and children on average are less sensitive than adults to chemical toxicity (i.e., the average ratio is greater than 1.0), but when they are not, the 3.16 kinetic component of UFH adjusts the adult human kinetic parameter to that of the infant or child for 91% of the chemicals tested (i.e.,

![Ratio of Child to Adult Value (1 Indicates Unity)](Image)

**FIG. 7.** Mean ratios of child to adult selected kinetic parameters (based on data from Renwick, 1998).
91% of the ratios are greater than 0.32, which is the division of a ratio of 1.0 by 3.16. Renwick et al. (2000) extended this work by analyzing chemicals that are eliminated primarily by a single pathway and reached similar conclusions.

Naumann (2001) investigated the adequacy of the 3.16 UFH subfactors using representative chemicals from five different therapeutic classes and different human populations. These populations included diseased individuals and some children. Subfactors were calculated as either the ratio of the mean and 2 standard deviations from a normal population for selected toxicodynamic and toxicokinetic parameters or the ratio of the mean of a normal population and the lower 95% of defined (by data) sensitive populations. For antidepressants these authors found that all toxicodynamic ratios varied between 1.1 and 1.7 while toxicokinetic ratios varied between 1.2 and 2.6 for normal populations and 2.9 to 7.5 for sensitive subpopulations. Of the 36 ratios studied for the latter group, 33 (92%) were less than the 3.16 kinetic component of UFH. For angiotensin inhibitors, all ratios were between 1.06 and 1.89 for dynamic variability while toxicokinetic ratios varied from 1.09 to 6.15; 149 of 156 ratios (96%) were less than the 3.16 kinetic component of UFH. For nonsteroidal anti-inflammatory drugs, toxicodynamic ratios were not determinable. Toxicokinetic ratios varied from 1.19 to 2.86 for the healthy population, while for several classes of sensitive individuals, ratios varied between 0.15 and 11.82. Of 53 ratios studied, 32 (60%) were less than 3.16. For cholesterol-lowering drugs, toxicodynamic ratios were not determinable. Toxicokinetic ratios varied from 1.3 to 2.2 for healthy populations and from 0.9 to 6.4 for various subpopulations including sensitive groups. Of 28 ratios evaluated, 24 (86%) were less than 3.16. For antibiotics, toxicodynamic ratios were not determinable. Toxicokinetic ratios varied from 1.3 to 4.7; of 10 ratios studied, 9 (90%) were less than 3.16. Thus, the default value of 3.16 for both toxicodynamics and toxicokinetics accounts for population variability most of the time, and these populations include diseased individuals.

Skowronski and Abdel-Ra'aman (2001) compared toxicokinetics and toxicodynamics among children, adults, and the elderly for six drugs. They estimated ratios of adult mean kinetic parameters to corresponding lower 95% kinetic values for children and the elderly. Toxicokinetic ratios between children and adults varied between 0.6 and 3.7; the lone toxicodynamic ratio was 1.2. The authors estimated the composite uncertainty factor using these comparative kinetic data and the IPCS default value for toxicodynamic variability. All composite values were less than a default value of 10-fold.

Ginsberg et al. (2002) evaluated child to adult toxicokinetics differences from a database derived from the therapeutic literature. The database was robust enough to demonstrate comparisons between adults and children of differing ages, for example, premature and full-term neonates, newborns (1 week to 2 months), and early infants (2 to 6 months). The database consisted of information on a number of cytochrome P450 pathways, certain phase II conjugation reactions, and renal elimination for 45 drugs. Results indicated that premature infants had on average about a four-fold longer half-life than adults. For full-term neonates and newborns, this average difference was about two-fold. Differences in average clearance were somewhat smaller among these groups, being less than two-fold in all cases. After 6 months, half-lives for these drugs in children were often found to be shorter than corresponding half-lives in adults; clearances were correspondingly higher. Half-lives and clearances were more variable for different types of chemicals. For example, for the CYP1A2 substrates caffeine and theophylline, the average half-life was approximately nine-fold greater for full-term neonates and about four-fold for newborns when compared with adult values.

**DISCUSSION**

Questions have been raised about whether current regulatory approaches to limiting chemical exposures are adequate to protect children from toxicity. Lead is often cited as an example of failed regulation due to insensitive animal testing. However, if lead were regulated on the basis of its developmental toxicity in laboratory animals, its action level would be much lower than it is at present, even without the addition of a child-protective safety factor (Plunkett, 1999).

Laboratory animals can be useful predictors of chemical hazards to humans whether they pose threats to children or adults. As more fully discussed in Scheuplein et al. (2002), growth and development are compressed into a shorter period in animals, which makes animal testing inherently more difficult. However, similar developmental events occur in both humans and laboratory animals and testing that covers the full period of animal development can reasonably be considered an appropriate surrogate for human development. It is likely that the weakest systems in the animal will be challenged sufficiently by the high doses tested to compensate adequately for compressed exposure and maturation periods. As long as the toxicity testing framework includes studies that encompass the developmental period and include systemic toxicity endpoints as appropriate, there is no reason to expect that significant human health hazards would not be detected by appropriate animal models. Overall, there appears to be a reasonable concordance among species for developmental toxicants and appropriate protocols compensate for differences in maturation rates. Additionally, testing guidelines appear to capture adequately a significant proportion of potential critical
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effects, and tiered systems of testing offer increasingly
detailed data sets that provide information on the
developing animal. Furthermore, although the data avail-
able to demonstrate the adequacy of UF_D are not exten-
sive, studies suggest that their use protects against the
likelihood that toxicity occurs at a lower dose or for an-
other effect than those tested most of the time.

When the aim is to identify and rank chemicals as po-
tential developmental threats in order to establish pri-
orities for further testing, various developmental tox-
ecology screening protocols are available and should be
used. Comprehensive developmental toxicity testing of
all chemicals is unnecessary (e.g., inhalation exposure
of hydrochloric acid). Consideration of screening test
results and the likely nature and extent of exposures
should identify substances that need further testing.
When more detailed knowledge of potential hazard is
needed, current toxicity testing protocols adequately
detect potential developmental toxicants as long as
studies are conducted in animal models that cover the
full developmental period in humans and the appropri-
te endpoints are measured.

Testing guidance has evolved over the past 2 decades
and, at present, approaches to hazard characterization
include in utero exposures. Evaluation of results from
well-designed and conducted developmental toxicity, re-
productive toxicity, and longer term repeat-dose studies
and integration of those results with appropriate uncer-
tainty factors is a reasonable and appropriate science-
based approach to limiting risks to the developing
human.

The EPA risk assessments include the use of un-
certainty factors when identifying criteria for limiting
chemical exposures. Those factors are designed to ac-
count for differences in susceptibility within and among
species and to compensate for limited data availability,
when necessary. Proposals have been made to use
an additional 10-fold uncertainty factor for the extra
protection of children when estimating safe exposure
limits from a database that is inadequate to determine
whether children are more sensitive to a chemical’s tox-
icity than adults. Use of such an additional uncertainty
factor, as is currently stated by the Food Quality Pro-
tection Act (FQPA) for pesticide safety evaluations, is
meant to address the same issues already addressed by
the EPA’s database uncertainty factor, UF_D, with addi-
tional issues related to exposure uncertainty. The EPA
further states that the use of the FQPA factor should be
modified when UF_D has already been used (U.S. EPA,
2002; Fenner-Crisp, 2001). Based on the data presented
here, the authors agree with this position.

Drawing conclusions about the adequacy of UF_H, the
uncertainty factor used to account for intrahuman vari-
ability, in terms of its ability to protect children from
environmental chemical exposures on the basis of the
modest data available is somewhat challenging. How-
ever, the studies reviewed here suggest that UF_H pro-
tects sensitive groups most of the time. Virtually all of
the studies available suggest that a high percentage of
the population, including children, is protected by using
a 10-fold uncertainty factor for human variability or by
using a 3.16-fold factor for either toxicokinetic or tox-
codynamic variability (Table 4). Based on specific com-
parisons for newborns, infants, children, and adults, the
percentage of the population protected is between 67
and 100, with the studies in larger populations that in-
clude sensitive individuals suggesting that the value is
closer to 100%. That percentage can be as low as 60 for
other sensitive populations, including those with severe
disease.

Where available, quantitative analysis of the extent
of toxicodynamic and toxicokinetic variability among
humans indicates that relying on a default value of 10
to compensate for variability among humans, including
that due to age, and on a default value of 10 to com-
pensate for a limited toxicity database, when necessary,
is adequate to protect most of the people—including
children—most of the time. Perhaps the strongest stud-
ies from which to draw reasonable and general con-
cclusions are those of Renwick and Lazarus (1998) and
Hattis et al. (1999a,b). Both groups of investigators
worked from large databases that included both kinetic
and dynamic parameters and evaluated normal and
sensitive populations, including children (Table 4). The
conclusion of both groups is that a UF_H of 10 is likely to
protect 99.9% or more of the population, and this pop-
ulation includes children. Because UF_H is applied to a
value in the low end of the distribution of human sen-
sitivities (i.e., a NOAEL), its use actually covers total
human sensitivity variations of 100 to 1000 times and
not 10 times, as is often thought. Furthermore, while
our presumption that UF_D and UF_H are independent
seems reasonable, this presumption may not be rea-
sonable for the two UF subfactors. If this assumption
is not reasonable, then multiplying factors together, as
is commonly done, is likely to introduce added conserv-
atism. Thought about in that context, the combined
likelihood that UF_H and UF_D are adequate to protect
children from unanticipated chemical toxicity appears
very probable.

Taken together, information on the relative sensiti-
vities of children and adults, on the sensitivity and speci-
ficity of toxicity testing protocols, and on the extent to
which current uncertainty factors compensate for in-
creased sensitivities and limited data suggests that the
use of additional uncertainty factors to limit envi-
ronmental chemical exposures is unlikely to provide sig-
ificantly greater protection to children over 6 months
of age. The same conclusion might not always hold true
for children younger than 6 months of age in the absence
of adequate developmental or systemic toxicity testing.
However, while younger children are often more sen-
sitive to toxicity than older children or adults, so are
younger laboratory animals. Thus, appropriate in utero
and early neonatal toxicity testing will compensate for any additional early sensitivity. Developmental and reproductive toxicity testing protocols such as those recommended by the EPA, FDA, and OECD are useful for characterizing toxicity in developing animals and for assessing risks to children that might arise from in utero and postnatal exposures.

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REFERENCES


