New Approaches in the Derivation of Acceptable Daily Intake (ADI)

INTRODUCTION

Current methods for estimating human health risks from exposure to threshold-acting toxicants in water or food, such as those established by the U.S. Environmental Protection Agency (U.S. EPA, 1980[1]; Stara *et al.*, 1981[2], the Food and Drug Administration (FDA) (Kokoski, 1976),[3] the National Academy of Sciences (NAS, 1977),[4] the World Health Organization (WHO, 1972)[5] and the Food and Agricultural Organization (FAO) (Bigwood, 1973[6]; Vettorazzi, 1976,[7] 1980[8]; Lu, 1983[9]), consider only chronic or lifetime exposure to individual chemicals. These methods generally estimate a single, constant daily intake rate which is low enough to be considered safe or acceptable. This intake rate is termed the acceptable daily intake (ADI).

Two problems with this approach have been recognized (Krewski *et al.*, 1984).[10] The first problem is that this method does not readily account for the number of animals used to determine the appropriate "no-observed-effect level." (NOEL). For example, if a chemical has a NOEL based on 10 animals and a similar NOEL based on 100 animals, the risk assessor will often choose the NOEL based on the larger study because it may appear to yield greater confidence in the resulting ADI. However, if these NOELs were for different chemicals, similar ADIs might be derived even though one would be associated with less confidence. It would be useful

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if the number of animals used to determine the appropriate NOEL could in some way affect the value of the resulting ADI, in addition to the level of confidence. The second problem with the current approach is that the slope of the dose-response curve of the critical toxic effect is generally ignored in estimating the ADI. Many scientists have argued that the slope should directly affect the resulting ADI (with steep curves presumably yielding higher values) because the threshold is more quickly obtained.

The purpose of this text is to illustrate both a revised approach to estimate ADIs with all toxicity data which includes methods for partial lifetime assessment, and novel methods for ADI estimation with quantal or continuous toxicity data. The latter method addresses to a degree the common problems with the current approach. The development of these methods can be found in U.S. EPA documents 600/9-84-008 and 600/9-84-014a U.S. EPA (1984a,b)[11,12]; these methods are also described by Stara *et al.* (1985a,b),[13,14] Crump (1984),[15] and Dourson *et al.* (1985).[16]

GRAPHIC APPROACH

Health risk assessments require evaluation of toxicity data derived from different species, different doses, different exposure durations, varied endpoints, and varied quality. This variety makes the health risk assessment extremely difficult. Therefore, it is valuable to have all toxicity data displayed simultaneously, if possible.

Toxicological data are defined in this discussion as quantal, continuous, or graded. Quantal data specify the number of animals affected as a function of dose rate (e.g., mg/kg bw/day) for a single type of effect. The numbers of animals with tumors or that die from a chemical exposure are examples. Quantal data are often reported as an incidence (percent response) and, thus, can be used to construct a dose-response curve. Continuous data represent the change in some measured value of a biological indicator (e.g., organ weights, triglyceride levels in the liver, and serum enzyme measurements) as a function of dose rate. Continuous data can be used to construct a dose-effect curve. Graded data specify the form or severity of adverse effects as a function of dose rate without reference to the number of animals affected or to a continuous

measure of one parameter. Graded data often are presented as categories (liver necrosis, lung lesions) or as judgments of severity. Fatty infiltration of the liver, single-cell liver necrosis, and liver necrosis are examples of sequence of severity judgments.

A graphic method is presented for this purpose. After thorough evaluation of the literature, toxicity data on a particular chemical might be summarized by five variables: 1) human equivalent dose rate (mg/day), 2) human equivalent exposure duration (years), 3) ranking of effects, 4) study quality and usefulness, and 5) target organs. In this discussion, human equivalent doses are calculated from animal doses by dividing the animal dose in mg/kg/day by the cube root of the ratio of human weight (70 kg) to animal weight in kg (w):

$$\sqrt[3]{\frac{70}{w}},$$

and multiplying the resulting dose by the assumed human body weight of 70 kg. All data on duration of exposure are expressed as years of equivalent human exposure. This value is determined by dividing the experimental exposure duration by the assumed species lifespan, and then multiplying this fraction by the commonly assumed average human lifespan of 70 years. These simple conversions allow construction of a dose-duration graph in which all observed effects from all available studies can be compared on a roughly equal basis (see Figs. 1 and 2). These conversions are presented for illustrative purposes; other approaches which allow for comparison of parameters among studies could also be applied.

The toxicity data from all studies (including human) are assigned to categories based on the severity of the observed effects in the case of graded data, or on the statistical or biological significance in the case of quantal or continuous data. In the latter case, the classification of quantal or continuous toxicity data into severity categories represents a loss of information. This could be prevented by adding a third dimension of percent response or change in effect onto Figs. 1 and 2. Each of the effect severity levels described above is represented by a unique symbol (see Table I). The size of the symbol represents a judgment of the quality of the study

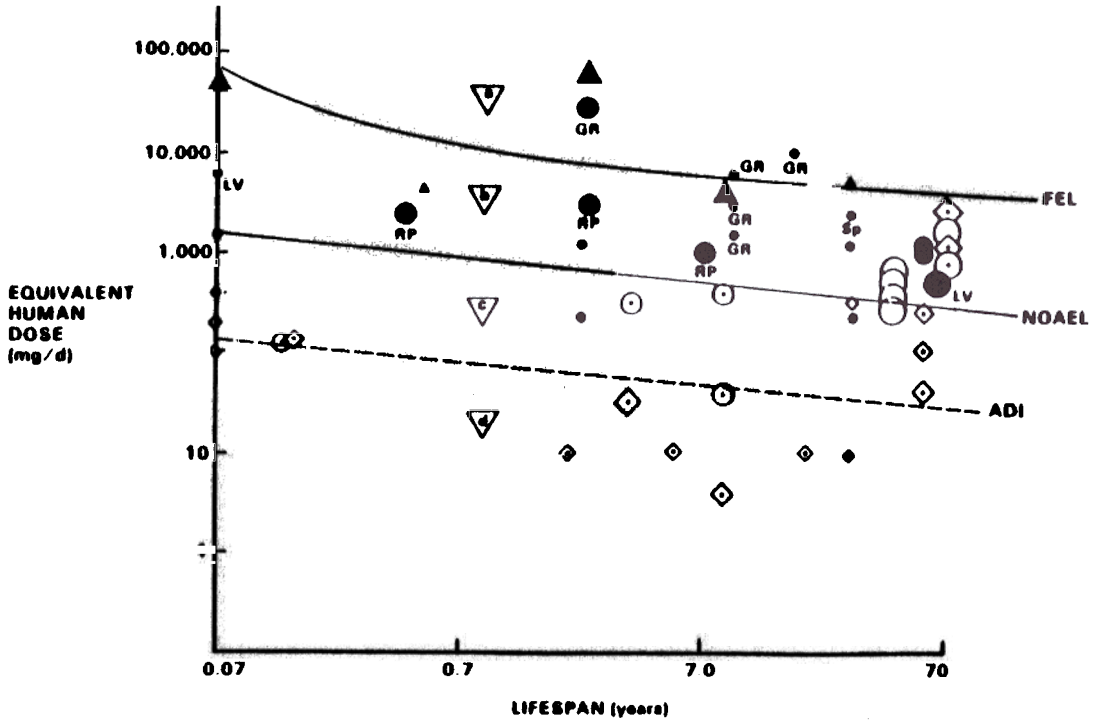

FIGURE 1 Effect-dose-duration plot of all relevant human and animal oral toxicity data for methoxychlor. Effect levels indicated by symbols are defined in Table 1. Animal doses have been converted by a body surface area factor to approximate the equivalent human dose. Dose durations are divided by the appropriate species lifespan to yield a fraction which, when multiplied by 70 years (the assumed average human lifespan), gives the corresponding position on the *x*-axis. Study usefulness is denoted by symbol size. Target organs are LV (liver), RP (reproductive organ), GR (growth reduction), and SP (spleen). The dose axis is divided into areas expected to cause either: gross toxicity and death (a), adverse effects (b), non-adverse effects (c), or no effects (d).

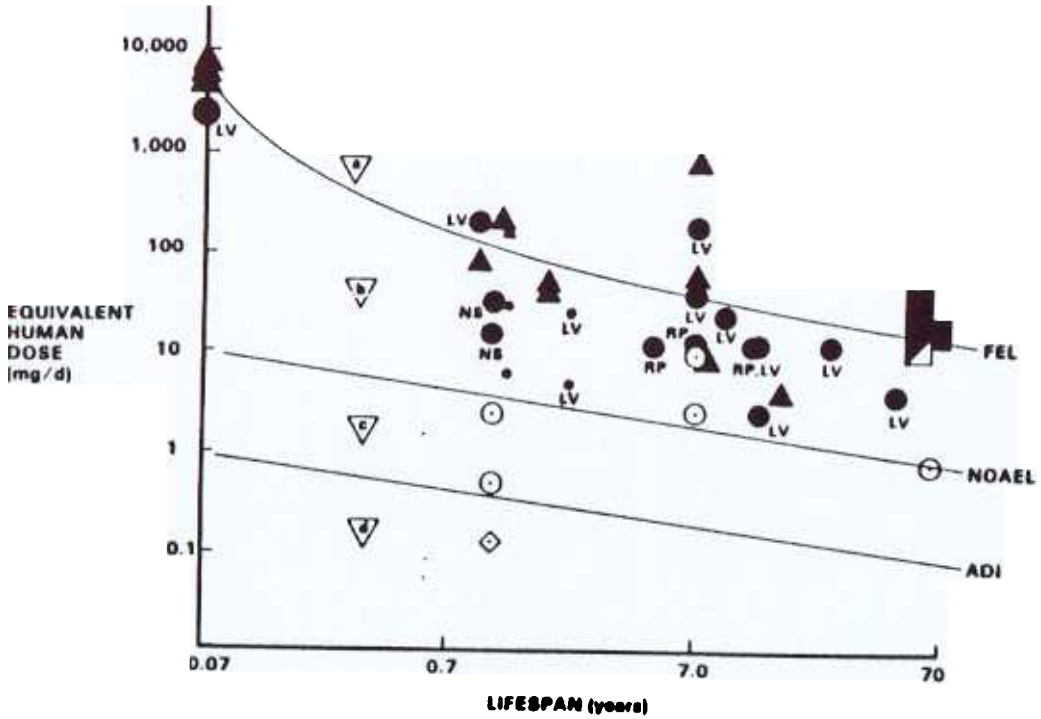

FIGURE 2 Effect-dose-duration plot of all relevant human and animal oral toxicity data for mirex. Effect levels indicated by symbols are defined in Table I with the addition of Cancer-Effect Level (■) and No-Observed-Cancer-Effect Level (□). Animal dose has been converted by a body surface area factor to approximate the equivalent human dose. Dose durations are divided by the appropriate species lifespan to yield a fraction which, when multiplied by 70 years (the assumed average human lifespan), gives the corresponding position on the *x*-axis. Study usefulness is denoted by symbol size. Target organs are LV (liver), RP (reproductive organ), NS (nervous system). The dose axis is divided into areas expected to cause either: gross toxicity and death (a), adverse effects (b), nonadverse (c), or no effects (d).

and its usefulness for risk assessment (with larger size denoting better quality or usefulness).

After graphic representation of all available toxicity data, a smooth boundary line is estimated (in Figs. 1 and 2 the line has been fitted by eye) which represents for any given time the highest no-observed-adverse-effect level (NOAEL) for which no lower adverse-effect level (AEL) is observed. Interpolation along this NOAEL curve can be performed to estimate the NOAEL for any desired partial-lifetime exposure. To obtain a corresponding acceptable intake, the estimated NOAEL is divided by an uncertainty factor. In Figs. 1 and 2 an uncertainty factor of 10 is used and accounts for the expected interhuman variability to the toxicity of a chemical (in lieu of chemical-specific data). Both the choice of the highest

TABLE I

Various effect levels and their definitions used in Figs. 1 and 2

Effect Level[a]	Symbol	Definition[b]
		Frank-Effect Level. That exposure level which produces unmistakable adverse effects, such as irreversible functional impairment or mortality, at a statistically or biologically significant increase in frequency or severity between an exposed population and its appropriate control.
AEL	●	Adverse-Effect Level. That exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.
NOAEL	○	No-Observed-Adverse-Effect Level. That exposure level at which there are no statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects are produced at this level, but they are not considered to be adverse.
NOEL	◇	No-Observed-Effect Level. That exposure level at which there are no statistically or biologically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

[a]Listed in order of decreasing severity.

[b]Adverse effects are considered as functional impairment or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (U.S. EPA, 1980).

NOAEL line (without lower AELs) and the suggested uncertainty factor of 10 are consistent with (and a logical extension of) previously established principles of the U.S. EPA (1980), the FDA (Kokoski, 1976), and the NAS (1977, 1980[17]) in the use of effect levels and uncertainty factors to estimate ADIs.

The basis of this method is empirical observations. As examples, data in Figs. 1 and 2 summarize the estimated human equivalent dose rates, exposure durations, effect levels, study suitability, and target organs from the toxicity data for methoxychlor and mirex, respectively. In these examples, the human equivalent dose rate (mg/day) is plotted versus the equivalent exposure duration (years) on a log-log scale using dose per body surface area and fraction of lifespan.

Upon close inspection of Figs. 1 and 2, certain general patterns are evident. The most prominent is that frank-effect levels (FELs) (indicated by solid triangles, see also Table I) decline in dose rate as exposure duration (i.e., fractions of life span) increases. This pattern is expected. A dose which is an LD_{50} in rats (and therefore a FEL) over a short duration should lead to at least 50% mortality after longer durations since the longer period includes the former. Note also the pattern of the solid circles (AELs) for a given duration. Solid circles tend to lie between FEL and NOEL or NOAEL values (indicated by diamonds or open circles, respectively). This pattern should also be expected since by definition AELs involve doses associated with adverse effects that are not as severe as those represented by FELs; whereas NOELs or NOAELs involve doses not associated with adverse effects. This pattern confirms the typical dose response, dose-effect, or dose-severity relationship expected at any given duration. The pattern of NOAEL or NOEL values versus fraction of lifespan is rather well defined with methoxychlor and somewhat less defined for mirex.

Another pattern that can be noted is that the maximum dose differences from study to study for any particular effect level (i.e., all of the NOELs) at a given general duration are often an order of 10 or less. This small difference appears remarkable considering that these data, although adjusted to represent humans, are from several different studies and include a variety of experimental animals as well as humans.

The slope for a toxicant is influenced by its mechanism of toxicity or its pharmacokinetics and, thus, the specific trends indicated by the methoxychlor or mirex data may not be representative of other chemicals. Zero or very small slopes suggest that the NOAEL line depends only on the dose rate, not the duration. Negative slopes may indicate a complex mechanism of toxicity. Some of the possible reasons for negative slopes are:

1) bioaccumulation of the chemical or its toxic metabolites;
2) accumulation of damage;
3) decrease in resistance to the toxic effects of the chemical as exposure continues (and the observed individuals grow older); or
4) multistage phenomena (whereby several organs or tissues must be compromised before overt toxicity ensues).

MATHEMATICAL MODEL APPROACH

Traditionally, NOAELs have been defined for quantal endpoints which have non-zero background incidences by choosing an experimental dose level which does not contribute to a statistically significant increase in incidence of adverse effects when compared to a control group. In parallel, NOAELs have been defined for continuous data by choosing an experimental dose level which does not constitute a significantly different mean value for a parameter indicating an adverse effect when compared to a mean value for a control group.

There are two limitations inherent in this approach. The first problem relates to the insensitivity of the current method for determining NOELs that use different numbers of animals, 0/10 vs. 0/100. For example, a dose-related trend in a parameter may suggest a deviation from the control incidence or mean value at an intermediate dose level(s) which is not statistically significant. This dose would be treated as a NOAEL. A statistically nonsignificant response could have biological significance especially when experimental groups are limited to small sample sizes and conclusions are extrapolated to larger populations.

The second limitation is related to the general lack of use of the slope of the dose-response curve. For example, the response in-

cidence or mean parameter measurement is expressed as the presence or absence of a statistically significant effect at discrete intervals (i.e., the experimental doses). The probability of response at a dose level between a lowest-observed-adverse-effect level (LOAEL) and a NOAEL is not addressed. Especially if doses are widely spaced, this could lead to considerable underestimation of the threshold dose.

The approach suggested here is not as subject to these limitations because it uses more of the dose-response or dose-effect curve. For example, an ADI might be calculated from a dose-response curve by defining an adverse effect as a risk level of more than a certain percentage above background, such as 10%. In this presentation 10% is chosen because many of the mathematical models in current use agree well at estimated risks in this range and because the better studies have sufficient numbers of doses and animals per dose to measure this level directly. The lower 95% confidence limit (CL) on the dose associated with this risk is then calculated. To obtain an ADI, the dose associated with this lower 95% CL might be reduced by a chemical-specific, species adjustment factor, or as in the case of Fig. 3 the cube root of the animal to human body weight ratio. Uncertainty factors might then be used to divide this adjusted value to yield the ADI.

In this review, uncertainty factors range between 10 and 100. The first uncertainty factor of 10 is interpreted as accounting for the expected variability in the general human population to the toxicity of the chemical. This uncertainty factor is consistent with previous U.S. EPA guidelines (U.S. EPA, 1980) as well as other guidelines [e.g., FDA (Kokoski, 1976); WHO (Vettorazzi, 1980); NAS, 1977, 1980]. The second uncertainty factor between 1 and 10 is thought to be necessary because the adjusted 95% CL corresponding to 10% response represents a LOAEL rather than a NOAEL. The use of this variable uncertainty factor is also consistent with previous guidelines (U.S. EPA, 1980). In this example, the choice for the value of this variable factor should depend on both the severity of the adverse effect (i.e., more severe effects yield a larger factor; U.S. EPA, 1980) and the slope of the dose-response, or dose-effect curve (i.e., shallower slopes also yield a larger factor). For example, a choice for this variable uncertainty factor of 1.0 should be associated with both a minimal adverse effect and a steep dose-response or dose-effect curve.

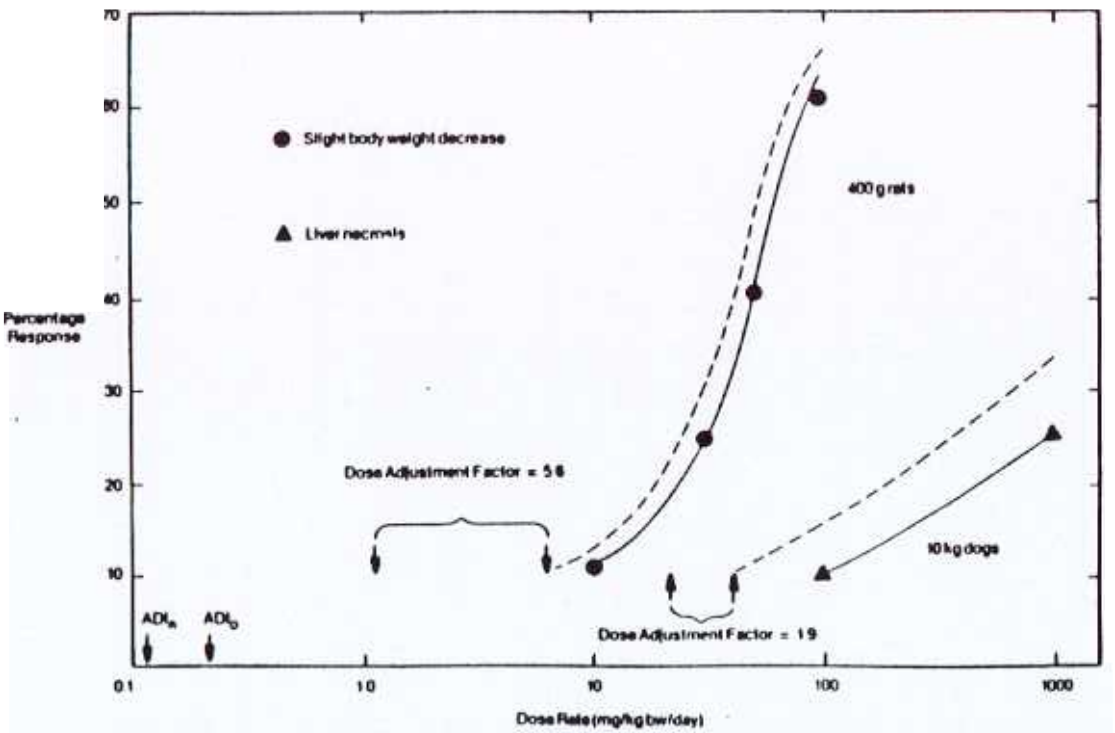

FIGURE 3 Hypothetical dose-response data for slight body weight decrease (●) or liver necrosis (▲) in rats and dogs, respectively. Solid lines indicate hypothetical data; dashed lines represent lower 95% confidence limits (CLs). An ADI has been estimated from the dog data (ADI_D) by a dose adjustment factor of 1.9 to the lower 95% CL and a 100-fold uncertainty factor. An ADI has been estimated from the rat data (ADI_R) by a dose adjustment factor of 5.6 and a 10-fold uncertainty factor. See text for additional explanation.

An example of this procedure is given in Fig. 3 which is a hypothetical plot of the percentage of rats responding with a slight body weight decrease of 5% versus dose rate or the percentage of dogs with liver necrosis versus dose rate. Hypothetical responses are indicated by solid lines; lower 95% Confidence Limits (CLs) on the dose rate are shown as dashed lines. The lower 95% CLs of the dose rates at a 10% response are adjusted by division by the cube root of the ratio of body weight between humans and rats or dogs. For rats of 400 g weight, this value is 5.6; for dogs of 10 kg weight, it is 1.9; both calculations assume a 70 kg body weight. To estimate an ADI from the rat data (shown in Fig. 3 as ADI_R), the adjusted lower 95% CL is divided by a 10-fold uncertainty factor to account for the expected variability in the general human population to the toxicity of a chemical in lieu of specific data, and an additional 1.0-fold factor because the effect is both minimally severe and has a steep dose-response slope. Thus, the total uncertainty factor is 10. To estimate an ADI from the dog data (shown in Fig. 3 as ADI_D), the adjusted lower 95% CL is divided by a 10-fold uncertainty factor to account for the expected human variability, as before, and an additional 10-fold uncertainty factor because the effect is both more severe than a slight body weight decrease and the slope of the dose-response is shallower. Thus, the total uncertainty factor is 100.

DISCUSSION

The primary advantage of the graphic method is that it provides a mechanism for viewing all of the data simultaneously resulting in an integrated profile of the toxicity of a compound. In addition, exposure duration-response trends, if present, are clearly delineated providing a possible strategy for estimating acceptable intakes for partial lifetime exposures.

The graphical method relies on a simple severity ranking system for data presentation (i.e., NOEL, NOAEL, AEL, and FEL). Obviously with such a simple system, effects within a given category (e.g., all AELs) may not be identical nor is it assumed that they are. Indeed, the critical toxic effect is often a function of exposure duration. In these cases the effects within a given category

will not be the same across time. However, the change in critical effect over duration (and, therefore, the change in effects within a category) is only of secondary regulatory importance. Since the NOAEL line is based on NOAELs of critical effects from all durations, the approach is consistent with the regulatory objective of guarding against any adverse effect. Moreover, while assumptions are needed in the process of extrapolation of dose and duration from animal studies to their human equivalent counterparts, this graphical method should enable regulatory scientists, at a glance, to judge the overall strength of evidence of toxicity, including the change of target organ as duration of exposure changes if desired, data gaps wherever they appear, and the resulting regulatory options that may be derived from such data.

Some of the limitations of this proposed procedure are that the development of the human equivalent dose is not based on much data and does not make provisions for incorporating interspecies differences in the metabolic patterns of dealing with different chemicals. That is, the method does not take into account differences in activation and detoxification, etc. It is also assumed that the log-log plot does not overly compress the data. The problems are particularly great for very short durations of exposure. Another limitation is that the time interval to develop pathologic signs after acute toxic insult may be more related to body size and pharmacokinetic parameters than to life span. In addition, most chemicals have scant data, and, thus, plots of these data may not yield useful generalizations.

The experiments used to develop the data base which was used to derive acceptable limiting concentrations for short durations were rarely, if ever, designed with that purpose in mind. Short-term experiments have been done in animals of many ages representing most phases of the total life span. Long-term experiments (of necessity) start with young animals and follow them through their life span. If there are age dependent differences in the sensitivity of the experimental species, these would confound the data sets used.

The proposed methods for estimating the 10% dose-effect or dose-response levels for continuous and quantal data, respectively, offer several advantages when compared with traditional methodologies (Crump, 1984). These advantages, as well as difficulties

with this approach, have been discussed (Dourson *et al.*, 1986[18]; Crump, 1986[19]). For example, with this new approach both the slope of the dose-response curve and the number of animals used in an experiment can affect the estimation of the ADI when quantal or continuous toxicity data are available. Another advantage of this method is that it can also estimate the health risk for supra-threshold exposure levels which might be useful for cost-benefit analysis. Several difficulties include finding appropriate data sets to model, choosing among equally good data sets that may yield different ADIs and, for cost-benefit analysis, assuming that a certain percentage response in an animal study is equivalent to a similar percentage response in humans.

In summary, the methods described for estimating ADIs utilize more of the available toxicity data than the current methodologies, and offer a consistent approach for possibly estimating health risks for less than lifetime toxicant exposure and perhaps health risks above the ADIs. They also address several of the criticisms of the current approach such as use of dose-response slopes and the number of animals tested in defining NOELs. More work is needed, however, before either or both of these methods are accepted as the *status quo*.

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