Methods for Assessing the Effects of Mixtures of Chemicals
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Risk Assessment Is a Developing Science: Approaches to Improve Evaluation of Single Chemicals and Chemical Mixtures

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ABSTRACT

The Environmental Criteria and Assessment Office of Cincinnati, of the US Environmental Protection Agency, is engaged in the development of risk assessment guidelines and methodologies as part of the regulatory programme of the Agency. The primary goal of the Agency was originally to accurately assess the effects of human exposure to single chemicals in a single medium (e.g. water) over a human lifetime. Assessment methods for estimating low-dose lifetime cancer risk for known or suspected human carcinogens, or long-term acceptable daily intake (ADI) levels for threshold-acting toxicants, were developed and successfully applied to meet this need.

Now, there is an increasing need for methods which address more complex exposure situations, including multichemical, multiroute, and partial-lifetime exposures. Improvements in the current methods are also needed. Several issues now under study will be briefly described.

To convert animal dose to equivalent human dose, an approach based on body surface area is being considered as an improvement to current methods which use body weight. To assess effects of less-than-lifetime exposures, separate approaches for carcinogens and non-carcinogens are proposed. Existing low-dose extrapolation models have been modified to enable prediction of cancer incidence at a variety of exposure durations. For non-carcinogens, a graphical method for displaying dose–duration–effect relationships, which permits interpolation to any exposure duration, has been developed.

Methods for determining equivalent doses for different exposure routes (e.g. inhalation versus ingestion) are straightforward for chemicals with certain toxicological characteristics, but demand specific knowledge of pharmacokinetic

parameters for many others. Where exposure is by more than one route simultaneously, equivalent doses for each route are combined to predict the total dose or the overall hazard posed by the chemical. Where exposure is to several chemicals at once, the total hazard posed by the mixture must be estimated. For non-carcinogens, a scheme whereby doses are each adjusted for potency and then summed is proposed, except where the mixture constituents are known to act by dissimilar mechanisms. For carcinogens, a form of risk additivity is proposed.

High-risk subgroups which may be present in the exposed population deserve special consideration, especially where a complex chemical mixture may elicit a larger-than-normal percentage of hypersensitive reactions. A rating scheme is proposed whereby the presence of individuals known to be more highly susceptible can be used to weight the final assessment. The uncertainty factor currently used to account for interindividual variability is also being reassessed.

Finally, a scheme has been developed which assigns a numerical ranking between 1 and 10 to various toxic effects, according to their severity, and to the doses which cause these effects. These dose–effect rankings lend themselves to new methods of displaying and interpreting patterns of toxicity which may be useful in improving the calculation of ADIs. All of these new developments are part of a continual effort to improve USEPA risk assessments.

1 INTRODUCTION

For more than four years, the Environmental Criteria and Assessment Office, Cincinnati (ECAO-Cin) of the US Environmental Protection Agency (USEPA) has been involved in the development of risk assessment guidelines and methodologies to be used in deriving 'safe' levels or incremental risk levels as part of the regulatory programme of the Agency. This work is being done in relation to the implementation of various legislative acts; for example, the 65 Ambient Water Quality Criteria documents were mandated by the Clean Water Act of 1977. The USEPA is required to enforce the following Acts:

Clean Water Act, 1977, and as amended, 1981
Safe Drinking Water Act, 1974, and as amended, 1977
Marine Protection, Research, and Sanctuaries Act, 1972
Clean Air Act, 1963, and as amended, 1966, 1970, 1971, 1977
Federal Insecticide, Fungicide, and Rodenticide Act, 1947, and as amended, 1972, 1975, 1978, 1980
Toxic Substances Control Act, 1976
Resource Conservation and Recovery Act, 1976
Comprehensive Environmental Response, Conservation and Liability ('Superfund') Act, 1980

Initially, the efforts under several of these legislative mandates focused on risk assessments of single chemicals involving primarily the oral route of exposure,

especially through water media. However, the methodological approaches are being continually expanded to evaluate other routes of exposure (i.e. inhalation and dermal) as well as to examine simultaneous exposure to chemical mixtures by various routes. The current status of development of all such guidelines is given in Table 1.

The development of the methodology used by ECAO-Cin for assessing the human health effects of environmental contaminants is an on-going process, continuously being revised as improvements are made in the science of risk assessment, as ways are found to apply various principles or developments in toxicology and related fields to the problems of risk assessment, and as the needs of the Agency change or expand. The evolution of our current approach can be traced through several publications (Stara *et al.*, 1980, 1981, 1983; USEPA, 1980, 1983a, b). Each of these publications reflects changes that have been made or are being considered in an attempt to improve methodological procedures or rationale. ECAO-Cin has also created a computer data base containing all available quantitative toxicity studies on USEPA priority pollutants to ensure that all data are considered in risk assessments and to assist in the development of new risk assessment models.

One pattern that is consistent in all of these developments is that the Agency is being asked to provide increasingly precise answers to increasingly difficult questions. Early in the development of regulatory toxicology, the goal was to define a 'safe' exposure for a given chemical. Now, we realize that absolute safety may not be attainable, and the problem is to define risks not only for single compounds but for mixtures of compounds. While we are not always able to answer these difficult questions, we are attempting to use as much of the relevant data base as possible in some reasonable and consistent fashion.

The goal of this review is not to attempt to detail all methodological changes that are being considered, but to focus on a brief summary of our current approach and on major and significant revisions that may substantially improve our ability to assess the hazard of environmental pollutants. Most recently our office is in the process of examining several key issues, including the following:

(1) interspecies dose conversion;
(2) less-than-lifetime exposure;
(3) pharmacokinetic parameters in route-to-route conversion;
(4) multiple-route and multiple-chemical exposures;
(5) high-risk (sensitive) subgroups;
(6) rating scheme for the severity of effects.

Each of the proposed revisions described in this paper is based on the proceedings of several workshops and represents the work of many scientists both within and outside the Agency. Many of the details were omitted for the sake of brevity in an attempt to provide a clear overview.

Table 1 Status of methodology development for health risk assessment by the USEPA

Exposure duration	No. of chemicals	Route of exposure						
		Systemic toxicants			Carcinogens			Both effects[a] (all routes)
		Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	
Acute	Single	Preliminary	Preliminary	None	Preliminary	Preliminary	None	Considered separately[b]
	Multiple	Preliminary	None	None	None	None	None	None
Subchronic	Single	Preliminary	Preliminary	None	Preliminary	Preliminary	None	Considered separately[b]
	Multiple	Preliminary	None	None	None	None	None	None
Chronic	Single	Published	Firm	None	Published	Published	None	Considered separately[b]
	Multiple	Preliminary	None	None	Preliminary	Preliminary	None	Considered separately[b]

[a] Includes single chemical which causes both systemic toxicity and cancer as well as mixtures of toxicants and carcinogens.

[b] Cancer and systemic effects treated as if they are independent.

Published = peer and public reviewed, published in *Federal Register* and open literature, previous/current use by the Agency. Firm = peer reviewed draft, not in use by the Agency. Preliminary = non-peer reviewed working draft, not in use by the Agency.

2 CURRENT METHODOLOGY

A complete set of health-risk assessment guidelines were reviewed and published by the Agency and used for deriving Ambient Water Quality Criteria for the protection of human health (USEPA, 1980). Under these guidelines, the risk associated with exposure to a particular chemical agent is defined according to the type of effect induced. It is the consensus of scientists and the National Academy of Sciences (NAS, 1977) that non-carcinogenic effects can be considered to be threshold phenomena, whereas carcinogenic effects are considered non-threshold phenomena. Biologically, threshold represents a no-effect level explained by an organism's resistance or sum total of defence mechanisms in the face of a toxicological challenge. In contrast, chemical carcinogenesis may result from the interaction of a single genotoxic molecule with the cell's DNA. The methodological approaches used for assessing risk from these two types of effects are described in the following sections.

2.1 Cancer (Non-threshold Effects)

After the decision has been made that a compound has the potential to cause cancer in humans and that adequate dose–response data exist, the criterion or water concentration associated with a given lifetime carcinogenic risk is estimated. The data used for quantitative estimates are of two types: (1) lifetime animal studies; and (2) human studies where excess cancer risk has been associated with exposure to the agent. The procedure for deriving the water concentration from animal studies involves the modified multistage model developed by Crump (1981) and certain techniques developed by the USEPA (1980).

If human epidemiological data with sufficiently valid exposure information are available for the compound, the data are analysed by alternative procedures which assume a linear dose–response relationship. If the epidemiological data show no significant carcinogenic effect when positive animal evidence is available, it is assumed that a risk exists but is smaller than could have been observed in the epidemiological study. An upper limit of the cancer incidence is calculated, assuming that the true incidence is just below the level of detection in the epidemiological studies.

Both of these procedures yield estimates of the slope of the dose–response curve in the low-dose region, called the cancer potency. The estimated human potency, B, is derived directly when adequate epidemiological data are available. When animal studies must be used, the human potency estimate is calculated as follows. The upper 95 % confidence limit on the animal potency, q_1^*, is estimated by fitting the linearized multistage model to the animal data. Then q_1^* is adjusted for exposure duration and species differences to give the estimated human

potency [in $(mg/kg/day)^{-1}$] (USEPA, 1980):

$$B = \frac{q_1^* (70/W_A)^{1/3}}{(l_e/L_e)(L_e/L)^3} \tag{1}$$

where

q_1 = animal potency $(mg/kg/day)^{-1}$
70 = assumed human weight (kg)
W_A = animal weight (kg)
l_e = length of exposure
L_e = length of experiment or observation period
L = lifespan of the animal.

The cube root of the ratio of body weights adjusts for species differences (see section 3.1), and the factor l_e/L_e adjusts the actual dose to a daily dose averaged over the length of the experiment. The third factor, $(L_e/L)^3$, adjusts the potency to reflect lifetime risk from lifetime exposure to the carcinogen. When the animal experiment is partial lifetime, this adjustment is necessary to allow for positive responses that would have occurred had sufficient time been allowed for the tumours to develop (Druckrey, 1967).

After the slope that estimates carcinogenic potency in humans has been calculated, the intake rate (I, in mg/day) associated with a specific human lifetime risk (usually 10^{-5} or 1 in 100 000) is determined:

$$I = \frac{70(10^{-5})}{B} \tag{2}$$

The ambient water quality criterion (C, in mg/l) is derived by a straightforward calculation:

$$C = \frac{I}{2 + [(0.0065)(\text{BCF})]} \tag{3}$$

The assumed average daily consumption of water and fish for a 70-kg man is 2 litres/day and 0.0065 kg/day, respectively. BCF is the average fish bioconcentration factor of the chemical (in litres/kg).

2.2 Toxicity (Threshold Effects)

In developing guidelines for deriving criteria based on non-carcinogenic response, five types of response levels were considered:

(1) NOEL = no-observed-effect level
(2) NOAEL = no-observed-adverse-effect level
(3) LOEL = lowest-observed-effect level
(4) LOAEL = lowest-observed-adverse-effect level
(5) FEL = frank-effect level.

Adverse effects are defined as any effects that result in functional impairment and/or pathological lesions that may affect the performance of the whole organism or that reduce an organism's ability to respond to an additional challenge. Frank effects are defined as overt or gross adverse effects (severe convulsions, lethality, etc.).

These concepts are illustrated in Figure 1. They have received much attention because they represent landmarks that help to define the threshold region in specific experiments. Thus, if an experiment yields a NOEL, a NOAEL, a LOAEL, and a clearly defined FEL in relatively closely spaced doses, the threshold region has been relatively well defined. Such data are very useful in deriving a criterion. On the other hand, a clearly defined FEL is of little use in establishing criteria when it stands alone because such a level gives no indication of how far removed it is from the threshold region. Similarly, a free-standing NOEL has little utility because there is no indication of its proximity to the threshold region.

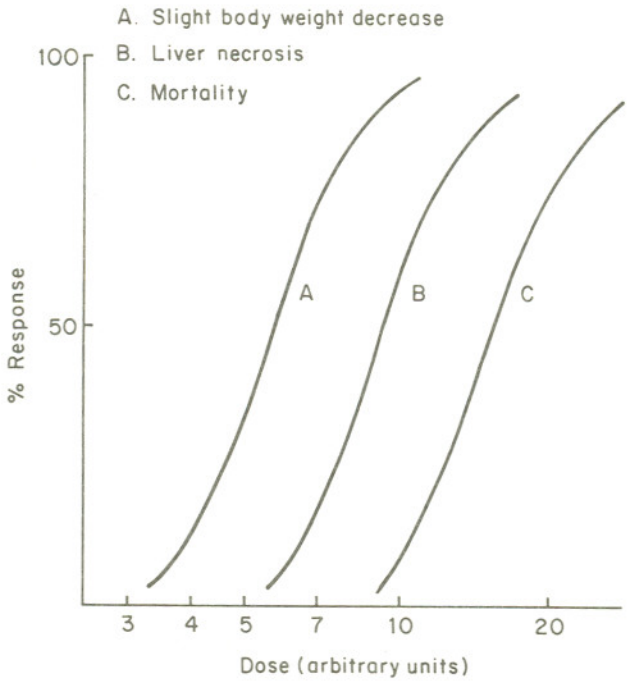

Figure 1 Response levels considered in defining threshold regions in toxicity experiments. Doses associated with these levels are as follows: 3 = NOEL; 4 = LOEL, NOAEL; 5 = NOAEL (highest); 7 = LOAEL; 10 = FEL; 20 = another FEL. Adapted from Stara *et al.* (1981)

Based on the preceding dose–response classification system, the following guidelines for deriving criteria from toxicity data have been adopted:

(1) A free-standing FEL is unsuitable for the derivation of criteria.
(2) A free-standing NOEL is unsuitable for derivation of criteria. If multiple NOELs are available without additional data on LOELs, NOAELs or LOAELs, the highest NOEL should be used to derive a criterion.
(3) A NOAEL, LOEL, or LOAEL can be suitable for criteria derivation. A well-defined NOAEL from a chronic (at least 90-day) study can be used directly, applying the appropriate uncertainty factor. For a LOEL, a judgement must be made as to whether it actually corresponds to a NOAEL or a LOAEL. In the case of a LOAEL, an additional uncertainty factor is applied; the magnitude of the additional uncertainty factor is judgemental and should lie in the range of 1–10. Caution must be exercised not to substitute FELs for LOAELs.
(4) If, for reasonably closely spaced doses, only a NOEL and a LOAEL of equal quality are available, the appropriate uncertainty factor is applied to the NOEL.

In using this approach, the selection and justification of uncertainty factors are critical. The basic definition and guidelines for using uncertainty factors have been given by the NAS (1977). 'Safety factor' or 'uncertainty factor' is defined as a number that reflects the degree or amount of uncertainty that must be considered when experimental data in animals are extrapolated to man. When the quality and quantity of experimental data are satisfactory, a low uncertainty factor is used; when data are judged to be inadequate or equivocal, a larger uncertainty factor is used. In those cases where the data do not completely fulfil the conditions for one category—or appear to be intermediate between two categories—an intermediate uncertainty factor is used. Such an intermediate uncertainty factor can be developed based on a logarithmic scale (e.g. 32 being half-way between 10 and 100 on a logarithmic scale). See also Dourson and Stara (1983) for a more complete discussion of uncertainty factors.

Adjustment of a NOEL, NOAEL, or LOAEL by the appropriate uncertainty factor yields an ADI (in mg/day) which is specific to the exposure route used in the study. An ADI for ingestion is substituted for I in Equation (3) to derive an ambient water quality criterion.

3 IMPROVED RISK ASSESSMENT METHODS UNDER STUDY

The revision of existing guidelines and the development of new assessment methods will improve our ability to assess hazards posed by environmental chemicals. Improvement to the guidelines must include the updating, based on new scientific findings, of approaches common to single and multiple chemicals, and the resolution of issues more specific to exposures to mixtures of chemicals.

Several issues currently under study are discussed below. Figure 2 shows the relationship of each of these issues to the overall risk assessment process.

3.1 Interspecies Dose Conversion

One of the most challenging aspects of regulatory toxicology is to develop a reasonable approach for converting doses given to experimental animals to equivalent human doses. This conversion is based on models for equitoxic dose, i.e. the exposure measure which elicits the same severity of toxic response in all species. For carcinogens, the current approach follows the assumption that a dose expressed as mg chemical per m^2 surface area is equitoxic across species. If the exposure is given in units of mg chemical per kg body weight, the equitoxic model can be written as follows:

$$d_H = (d_A \times W/70) \div (W/70)^{2/3} = d_A (W/70)^{1/3} \quad (4)$$

where d_H and d_A are the human and animal daily exposure levels (mg/kg), 70 is the assumed human weight (kg), and W is the animal weight (kg). This equation also depends on the assumptions that the effective concentration of a chemical is directly related to metabolic rate, that metabolic rate is proportional to body surface area, and further that surface area is proportional to body weight raised to the 2/3 power.

The assumption of mg per surface area as the equitoxic dose is empirically supported by the work of Freireich *et al.* (1966). Based on quantitative comparisons of the toxicity (LD_{10} or maximum tolerated dose) of 18 anti-neoplastic agents to mice, rats, hamsters, dogs, monkeys, and men, Freireich *et al.* (1966) suggested that the maximum tolerated dose was more constant across species when presented as weight of chemical per body surface area (mg/m^2) than when presented as weight of chemical per body weight (mg/kg). Other conversion factors based on differences in body weight have also been proposed (Dixon, 1976; Goldin *et al.*, 1973; Kresovskii, 1976). Some problems, however, can be encountered in the application of any of these factors. As pointed out by Rall (1969) in his discussion of the Freireich study, the compounds tested were 'generally not involved in variable drug metabolizing systems'. Consequently, species conversion factors based on surface area may not be the most appropriate method for all environmental chemicals.

Until recently, this species conversion factor has been used by the USEPA only for lifetime exposure to carcinogens, with species differences for non-carcinogens being considered as part of the uncertainty (safety) factor (Dourson and Stara, 1983). Now, however, the Agency is considering using this approach for both carcinogens and toxicants.

3.2 Risk Assessments for Less-than-lifetime Exposures

Currently, risk asessments have to be conducted for exposure periods ranging from very short term to full lifespan, including exposure periods which occur at

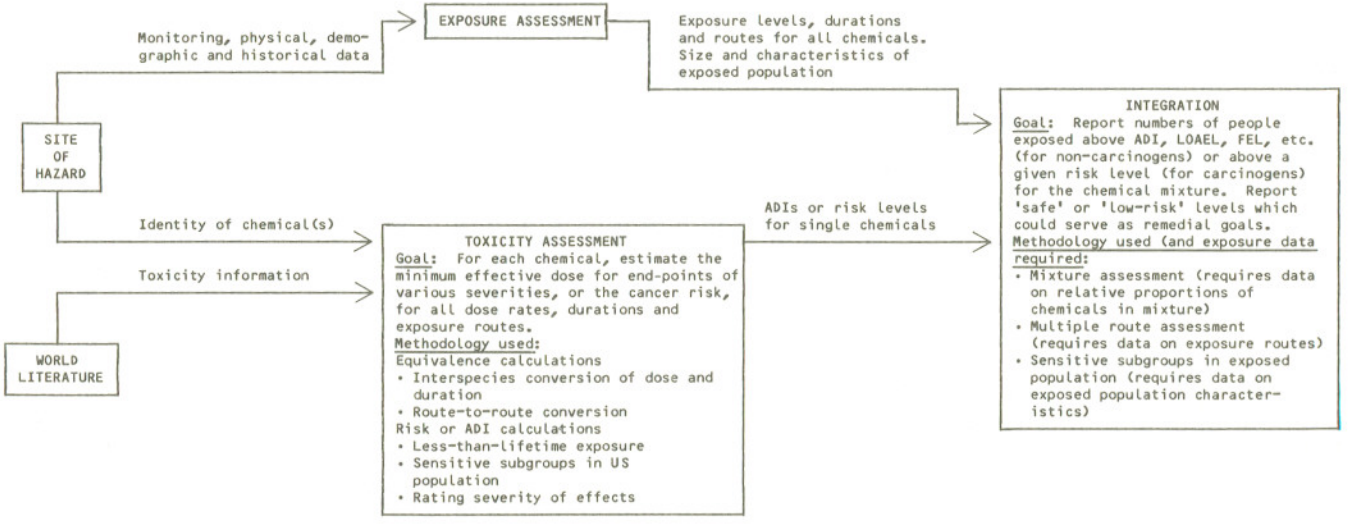

Figure 2 Information flow and methodology use in conducting site-specific multichemical health risk assessment, showing use of each methodology component under development by the USEPA

any period during the normal lifespan. USEPA methods currently in use only estimate risks from lifetime exposures. However, Crump and Howe (1983) have developed a statistical application of the original Armitage–Doll model to estimate risk of partial lifetime exposure to carcinogens. Their approach allows for intermittent and variable exposures as well as early-stage or late-stage carcinogens. ECAO-Cin is currently reviewing this approach.

Dose–duration gaps also exist in the available data for non-carcinogens. Both Weil (Weil and McCollister, 1963; Weil *et al.*, 1969) and McNamara (1976) have attempted to derive empirical relationships between long-term and short-term exposures and the resulting toxic effects. However, variations among chemicals in their analyses and the limited types of chemicals considered would seem to preclude the development of a single 'temporal correction factor' for toxic effects. Therefore, a chemical-by-chemical approach has been used. For each chemical under review, an effect–dose–duration plot is constructed, as shown for methoxychlor in Figure 3. Each symbol in the figure represents an experimental observation placed on the graph at the exposure duration and average daily dose of the experiment. The dose rate in the graph has been converted to an estimated human dose rate by the mg per surface area equitoxicity model, and the exposure duration has been converted to the equivalent fraction of the human lifespan. The larger symbols indicate greater confidence in the data. Depending on the consistency of the pattern, a statistical or judgemental (eye-fit) approach could be

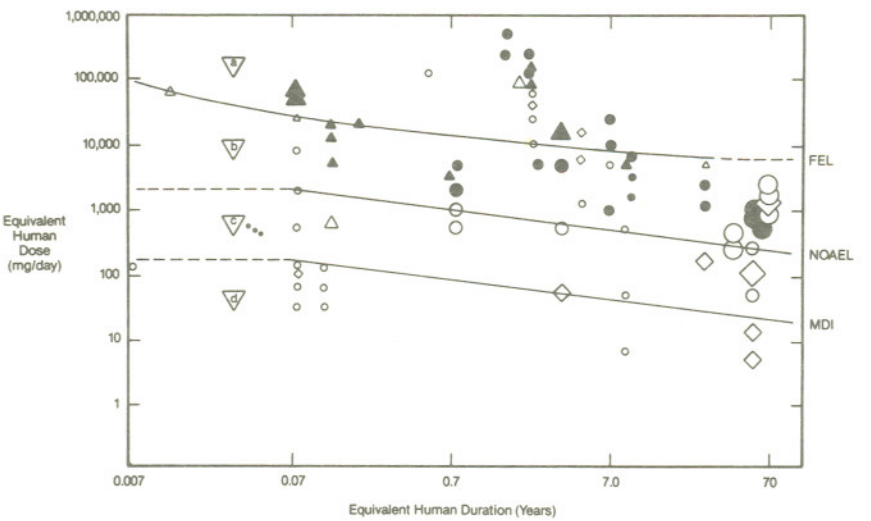

Figure 3 Effect–dose–duration plot for all relevant human and animal oral toxicity data for methoxychlor. (▲), FEL; (●), AEL; (○), NOAEL; (◇), NOEL. See text for explanation. From Dourson *et al.* (1985)

used to interpolate or extrapolate to exposure durations for which actual data are not available. In Figure 3, the judgemental approach has been used to divide the dose axis into areas expected to cause (a) gross toxicity or death, (b) adverse effects, (c) non-adverse effects, or (d) no effects. This tool permits the estimation of various types of effect levels corresponding to any exposure duration.

3.3 Pharmacokinetic Parameters in Route-to-route Conversion

Route-to-route extrapolation is necessary when data are available for one exposure route but unavailable for the route of interest. The Stokinger–Woodward model has been widely used for determining an oral ADI from inhalation data (Stokinger and Woodward, 1958). The USEPA Ambient Water Quality Criteria documents used this method to estimate ADIs for ingestion from available inhalation data, usually the threshold limit values (TLVs). According to the American Conference of Governmental Industrial Hygienists (ACGIH, 1981) threshold limit values 'refer to airborne concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse effect.'

The limitations brought out at a recent ECAO-Cin workshop (September, 1982) for this method included the following:

(1) Precisely measured absorption factors may be lacking.
(2) Hepatic metabolism may alter the circulating dose following oral exposure.
(3) The TLV may not be based on systemic toxicity.
(4) Temporal patterns of blood levels post administration are not considered.

The long-term goals identified include development of more sophisticated models which predict the concentration in the critical target tissue and account for transient blood levels from intermittent short-term exposure as well as steady-state levels from chronic exposures. In the interim it is proposed that extrapolation, especially from oral to inhalation and vice versa, is feasible under the following conditions:

(1) The chemical has a long elimination or inactivation half-time. With short half-times, intermittent exposures, such as those occurring with oral intake, may result in large fluctuations in concentration at the target organ, compared with inhalation exposure, where concentrations tend to be more stable (Figure 4).
(2) The systemic effects are identical for each exposure route.
(3) There are no effects at the portal of entry. Such effects are likely if the compound either is highly reactive or has a long residence time at the portal of entry.
(4) Removal, inactivation or activation of the compound before reaching the target organ does not vary substantially with exposure route (i.e. there is no 'first-pass effect').

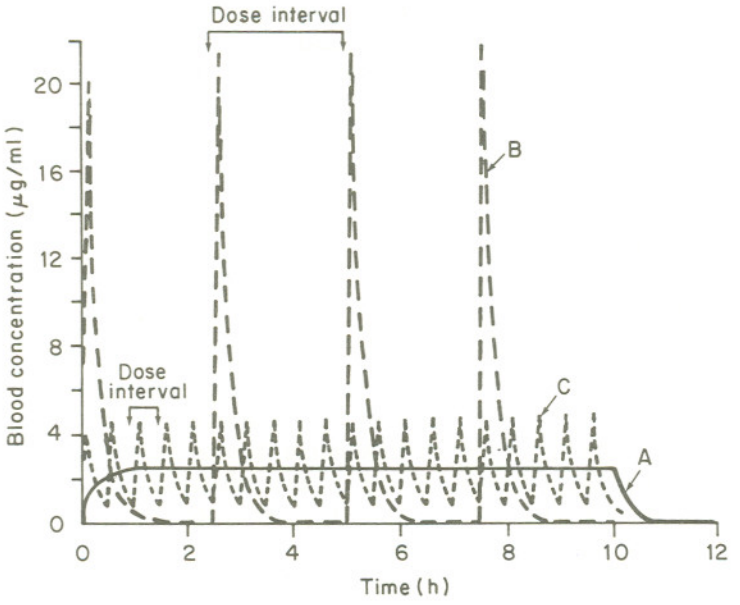

Figure 4 Theoretical blood concentration when equivalent total dose is administered over 10 hours by: inhalation (A, solid line); oral route, every 2.5 hours (B, long dashes); or oral route, every 0.5 hours (C, short dashes). Absorption half-time is 0.9 minutes and elimination half-time is 9 minutes. From Withey (1983)

(5) The relationship between the external and internal dose (i.e. the absorption factor) is known for each exposure route.

The Agency is, of course, aware that several complex pharmacokinetic models are available. While these models have proven useful in the pharmaceutical industry, sufficient data are not usually available on environmental or industrial toxicants to evaluate the model parameters. Nonetheless, some simple pharmacokinetic information such as approximate half-times is often available, and ECAO-Cin is studying the validity of using this type of data in route-to-route extrapolation.

4 MULTIPLE-ROUTE AND MULTIPLE-CHEMICAL ASSESSMENTS

4.1 Multiple-route Assessment

Since environmental contaminants may occur in or be transported by several environmental media (e.g. water, food, air, soil), humans may be exposed by two or more routes simultaneously. The three main portals of entry are the

gastrointestinal tract, lungs, and skin. For chemicals satisfying the conditions described in section 3.3, which permit route-to-route conversion of dose, absorbed doses are assumed to be additive among routes, as follows:

$$d_T = d_O r_O + d_I r_I + d_D r_D \tag{5}$$

where

d = dose (in mg/day)
r = absorption fraction
the subscripts O, I and D represent the oral, inhalation, and dermal exposure routes, respectively
d_T is the total absorbed dose.

The precise values of r are usually unknown; therefore, estimated values must often be assigned, according to knowledge about the type of substance and the exposure route. This approach further assumes there is no interaction between the exposures (e.g. the oral exposure does not alter inhalation rate or absorption), an assumption which has not yet been fully evaluated. The threshold then depends on the absorbed dose, regardless of route. The total dose thus obtained may be compared with the ADI for any exposure route (J) to give a hazard index (HI) for that chemical:

$$\mathrm{HI} = d_T/(\mathrm{ADI}_J \times r_J) \tag{6}$$

Where dose addition for multiple routes is inappropriate, as in the case where different effects are associated with each exposure route, but where a practical threshold (Th), such as a criterion or other type of safe level, has been established for each route, the HI may be determined as follows:

$$\mathrm{HI} = E_O/\mathrm{Th}_O + E_I/\mathrm{Th}_I + E_D/\mathrm{Th}_D \tag{7}$$

where E = exposure in units corresponding to the threshold. (For example, E_I and Th_I may be defined in terms of air concentration, rather than inhaled dose as in Equation (5).) A drawback to this approach is that threshold values for all needed routes frequently are not available.

If HI for a given compound is less than unity, no hazard is assumed to exist. If HI is greater than unity, a hazard is assumed, but the magnitude of the hazard is defined only in relative terms with respect to the ADI or the practical threshold for the chemical at hand. Although this approach does not define dose–response relationships, it would be possible, if sufficient data were available, to derive practical thresholds for a spectrum of effects (e.g. minimal effects on several organs, severe effects on several organs, reproductive dysfunction, behavioural effects, and mortality). If practical thresholds could be derived for such a spectrum of effects, the results of the hazard assessment would suggest not only if effects were likely to be seen but also what types of effects, if any, might be expected.

4.2 Multiple-chemical Assessment

The simplest mathematical models for joint action describe either dose addition or response addition. Dose addition, referred to as simple similar action by Finney (1971) and simple joint action by Bliss (1939), assumes that the toxicants in the mixture behave as if they were dilutions or concentrations of each other. Thus, the response elicited by the mixture can be predicted by summing the individual doses after adjusting for differences in potency. One means of adjusting doses for potency differences is to divide each dose by that chemical's ADI or threshold, as described above for the derivation of HI. For example, if the absorbed doses of the components of a two-chemical mixture are expressed in terms of the equivalent dose of chemical 1, the mixture dose, D, is

$$D = d_1 + d_2(\mathrm{ADI}_1 \times r_1)/(\mathrm{ADI}_2 \times r_2) \tag{8}$$

To determine whether any hazard is expected, D may be compared to the ADI for chemical 1. Equivalently, both sides of this equation could be divided by $\mathrm{ADI}_1 \times r_1$

$$D/(\mathrm{ADI}_1 \times r_1) = d_1/(\mathrm{ADI}_1 \times r_1) + d_2/(\mathrm{ADI}_2 \times r_2) \tag{9}$$

and the result compared to unity. From Equation (6), this suggests that dose addition can be used to calculate the total hazard index ($\mathrm{HI_T}$) for exposure to a mixture by summing the hazard indices for n, the number of toxicants of concern:

$$\mathrm{HI_T} = \sum_{i=1}^{n} \mathrm{HI_i} \tag{10}$$

Again, if practical thresholds for a spectrum of effects could be defined, $\mathrm{HI_T}$ could be calculated for each effect. The total index for the mixture is also compared to unity to determine qualitatively the existence of a hazard resulting from the total exposure. This type of approach has been recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1981).

Both Equations (7) and (10) imply that several subthreshold, ineffective exposures could have cumulative adverse effects. This suggestion is controversial, especially where the various thresholds are for dissimilar effects. For this reason, the ACGIH (1981) recommends against hazard addition when mixture components are known to act independently, i.e. on different target organs. Lacking any information on possible interactions, however, it may be necessary to presume that hazards are additive.

The other form of additivity, termed response addition (Bliss, 1939), assumes that the toxicants in a mixture act on different receptor systems and that the pairwise correlation of individual tolerances may range from completely negative ($r = -1$) to completely positive ($r = +1$). Response addition assumes that the response to a mixture of toxicants is completely determined by the responses to the components and the tolerance correlation coefficient. Taking P_3 as the

proportion of organisms responding to a mixture of two toxicants which evoke individual responses of P_1 and P_2,

$$P_3 = P_1 \text{ if } r = 1 \text{ and if } P_1 \text{ is } > P_2 \tag{11}$$

$$P_3 = P_2 \text{ if } r = 1 \text{ and if } P_1 \text{ is } < P_2 \tag{12}$$

$$P_3 = P_1 + P_2 \text{ if } r = -1 \tag{13}$$

$$P_3 = P_1 + P_2 (1 - P_1) \text{ if } r = 0 \tag{14}$$

Equation (14) can be generalized to calculate total expected response (P_T) for any number of chemicals, as follows:

$$P_T = 1 - \prod_{i=1}^{n} (1 - P_i) \tag{15}$$

It should be noted that response rates are not usually available for systemic toxicants, but are frequently estimated in studies on carcinogens, based on the methods described earlier in this paper.

A hypothetical example of values of P for five different chemicals (I–V) associated with a total of six effects of concern (A–F) is presented in Table 2. The goal is to estimate the expected incidence of response for each effect (PE_i), the combined incidence of response for each chemical (PC_i), and the cumulative incidence of adverse response in the population (P_T). Response addition can be used to estimate responses across effects or across chemicals. However, in each case a value must be chosen for the correlation coefficient, r, to determine which of Equations (11)–(15) should apply. For example, the correlation coefficient (r_E) for individual susceptibilities to different effects (such as various tumour sites) for chemical i must be known or estimated to calculate PC_i. Similarly, the coefficient (r_C) for individual susceptibilities to different chemicals is required for calculation of PE_i, and both are required in order to calculate P_T.

In many cases, the value of r_E will be positive; that is, effect A is more likely to be observed in an individual displaying effect B. This correlation may occur because the effects examined are closely related (e.g. lung adenoma and lung adenocarcinoma) or because tolerances for several dissimilar effects depend on the same factor, such as toxicant blood level. However, except where very closely related effects are involved, or tolerances are otherwise known to be strongly positively correlated, the conservative assumption is followed that $r_E = 0$. Thus, in Table 2, PC_i is calculated using Equation (15).

The correlation of individual tolerances to various carcinogenic chemicals (r_C) within the human population is not known. Some evidence suggests that cancer susceptibility in humans may be partly genetic. Furthermore, strain differences within a species in the susceptibility to chemical carcinogens also suggest a genetic component. Thus, a case probably could again be made for assuming that r is positive. Nonetheless, the degree of the correlation cannot be estimated and r_C probably varies for different carcinogens and systemic toxicants. Consequently, it

Table 2 Example of assessment of multiple toxicant/effects risk assessment

	Effects of concern						
Chemical	A	B	C	D	E	F	PC_i
I	2×10^{-2}			8×10^{-4}			2.08×10^{-2}
II		3×10^{-3}			1×10^{-3}		4.00×10^{-3}
III			4×10^{-2}			7×10^{-3}	4.67×10^{-2}
IV	5×10^{-3}			9×10^{-3}			1.39×10^{-2}
V		6×10^{-4}				6×10^{-3}	6.60×10^{-3}
PE_i	2.49×10^{-2}	3.60×10^{-3}	4×10^{-2}	9.79×10^{-3}	1×10^{-3}	1.30×10^{-2}	$P_T = 8.9 \times 10^{-2}$

From Stara *et al.* (1985).

seems reasonable to also assume that $r_C = 0$, as has been done in calculating PE_i and P_T in Table 2. This can be criticized as being somewhat conservative but it is certainly less conservative than assuming that $r = -1$. Assuming that $r = +1$ would probably underestimate the risk by reducing the number of respondents.

When $r = 0$, the probability of two or more effects occurring in one individual is the product of their individual probabilities. The purely quantal definition of risk under this method examines the number of responders but does not distinguish between single and multiple effects in a responding individual. This may have some justification in interpreting carcinogenic risk, for which it is most often used, because (a) risks at environmental contamination levels are usually small, thus the model approaches straight additivity of risk, and (b) the response is considered extremely severe and irreversible, so a yes-or-no evaluation seems appropriate. In practical terms, however, the occurrence of two tumours is more adverse to health than the occurrence of one. Moreover, co-occurrence of multiple toxic effects is obviously more severe than a single effect. Consequently, the use of Equation (15) is best justified when overall risks are small, and may be better justified for the calculation of cancer risk than of toxic risk. Where multiple effects are judged significant, however, their probability can be easily calculated and expressed as an additional factor to be considered.

5 HIGH-RISK (SENSITIVE) SUBGROUPS

A high-risk (HR) group or individual is one who will experience an adverse health effect from exposure to one or more pollutants significantly sooner or at a lower level than the general population because of the presence of predisposing factors. These factors include developmental stages (e.g. the developing embryo, the aged), nutritional deficiencies, disease states, genetic conditions, behavioural factors, and previous or concomitant exposures (Calabrese, 1978).

Risk to human health resulting from exposure to chemicals is estimated from human or animal data. Extrapolations from animal data involve making inferences from a healthy, properly fed, genetically homogeneous animal population to a human population which includes groups which vary in age, health, and nutritional status, and is genetically heterogeneous. Similarly, human data, when available, are often derived from a working population of healthy adults, usually male, or may consist of a TLV which is not systematically defined as a NOEL. Therefore, estimates of human health risk based on extrapolation from animal data or based on occupational data should incorporate some compensation for the HR individual.

The present guidelines for calculating an ADI for systemic toxicants from studies on the average animal or human include a 10-fold uncertainty factor to account for intraspecies variability, i.e. to extrapolate from the average to the sensitive human. However, the actual variability arising from the intrinsic and extrinsic factors listed above (developmental processes, age, disease, etc.) is not

known. When assessing risk from carcinogens, the upper 95 % confidence limit on the risk derived by the extrapolation procedure is used. This conceptually accounts for random variability associated with animals or individuals similar to those in the study population.

The 10-fold uncertainty factor for systemic toxicants is assumed to account for the general interindividual variations of a normal healthy adult population. Variability among other population groups may be much greater for developmental stages such as the embryo and neonate or for those with pre-existing diseases. Genetic variability is more difficult to identify but it is known that the human population is heterogeneous for the genetic and environmental factors that influence drug disposition. Wide interindividual variations in drug disposition have been well documented (Omenn and Motulsky, 1978). These genetic differences may affect responses to environmental chemicals and other environmental factors as well as to drugs. Several environmental factors such as smoking and diet, as well as chemicals, affect rates of drug elimination in human subjects. Differences in drug elimination rates can lead to differences in plasma drug concentrations and, thereby, increase or decrease the risk of toxicity.

At this time the magnitude of interindividual variation is not known for specific chemicals. Until this assessment is made it is important to consider the total number of individuals at high risk when exposure is defined for a specific population, such as that in the vicinity of an uncontrolled hazardous waste site. It is possible to identify HR groups for the chemicals in question on the basis of existing data and to assess their prevalence and sensitivity in order to account for the potential impact of a particular chemical mix on an exposed population.

Table 3 is an example of this approach for a typical waste site. The last column summarizes the potential number of HR individuals in a hypothetical population of 5000. An estimated 490 people (10 %) could be expected to be at high risk. This demonstrates that, for multichemical exposure, the total number of HR individuals could constitute a significant proportion of the population. Different chemical mixtures will result in different percentages at excess risk. This approach to the data facilitates comparisons among waste sites and gives more accurate information on the human health risks associated with a defined exposure scenario. Furthermore, specific information on the population around the site, such as day-care centres, aged populations, hospitals, or other institutions, would facilitate more accurate assessment of the risk.

6 RATING SCHEME FOR SEVERITY OF EFFECTS

As part of an effort to develop reportable quantities (i.e. that quantity of a chemical which, if released to the environment, must legally be reported) under 'Superfund' legislation, ECAO-Cin has been involved in the development of an approach to numerically rate the severity of adverse, non-carcinogenic effects (Table 4). This scheme for rating effects has been combined with another method

Table 3 Sensitive subgroups associated with a typical inventory of chemicals at waste sites

Chemical	Sensitive subgroup	Prevalence rate[a] (per 1000)	Number of sensitive individuals[b] in hypothetical population of 5000
Chlorinated ethanes (dichloroethanes)	CHD[c]	CHD: 24	120
	Liver condition	Liver condition: 20	100
	Pre-exposure to hepatotoxins		
	Embryo/foetus	Pregnant women: 21	105
Dichloroethylenes	CHD		
1,1,1-Trichloroethane	CHD		
	Liver condition		
	Pre-exposure to hepatotoxins		
Trichloroethylene	Liver condition		
Chloroform	CHD		
	Lung	Lung (bronchitis): 33	165
	Total		490

[a] Based on data collected by US National Center for Health Statistics.

[b] Some individuals may belong to more than one sensitive subgroup.

[c] Coronary heart disease.

Adapted from Erdreich and Sonich-Mullin (1983).

Table 4 Rating values for NOAELs, LOAELs and FELs used to derive reportable quantities based on chronic toxicity

Rating	Effect
1	Enzyme induction or other biochemical change with no pathological changes and no change in organ weights.
2	Enzyme induction and subcellular proliferation or other changes in organelles but no other apparent effects.
3	Hyperplasia, hypertrophy, or atrophy but no change in organ weights.
4	Hyperplasia, hypertrophy, or atrophy with changes in organ weights.
5	Reversible cellular changes: cloudy swelling, hydropic change, or fatty changes.
6	Necrosis, or metaplasia with no apparent decrement of organ function. Any neuropathy without apparent behavioural, sensory, or physiological changes.
7	Necrosis, atrophy, hypertrophy, or metaplasia with a detectable decrement of organ functions. Any neuropathy with a measurable change in behavioural, sensory, or physiological activity.
8	Necrosis, atrophy, hypertrophy, or metaplasia with definitive organ dysfunction. Any neuropathy with gross changes in behaviour, sensory, or motor performance. Any decrease in reproductive capacity. Any evidence of foetotoxicity.
9	Pronounced pathological changes with severe organ dysfunction. Any neuropathy with loss of behavioural or motor control or loss of sensory ability. Reproductive dysfunction. Any teratogenic effect with maternal toxicity.
10	Death or pronounced life-shortening. Any teratogenic effect without signs of maternal toxicity.

From Durkin and Colman (1983).

for rating the dose level and has been used to assess dose/severity patterns for ~ 250 non-carcinogenic compounds. The development of this rating scheme for effects and the combination of this scheme with a quantitative estimate of dose represent a potentially useful innovation in the analysis and assessment of chronic toxicity data and the estimation of ADI.

The effects rating scheme is based on a combination of biochemical, histological, physiological, and gross effects arranged in increasing order of severity. While the rating value assigned to each type of effect (RV_e) is essentially arbitrary, rating values of 1 to 3 or 4 have been generally regarded as no adverse effects, 5–7 as adverse effects, and 8–10 as frank effects. In an attempt to allow for the necessary scientific judgement, the description of the effects is intentionally limited in detail, and is not organ-specific.

The dose rating value (RV_d) for a given chemical is based upon the minimum effective dose (MED) transformed to values ranging from 1 to 10 as described in

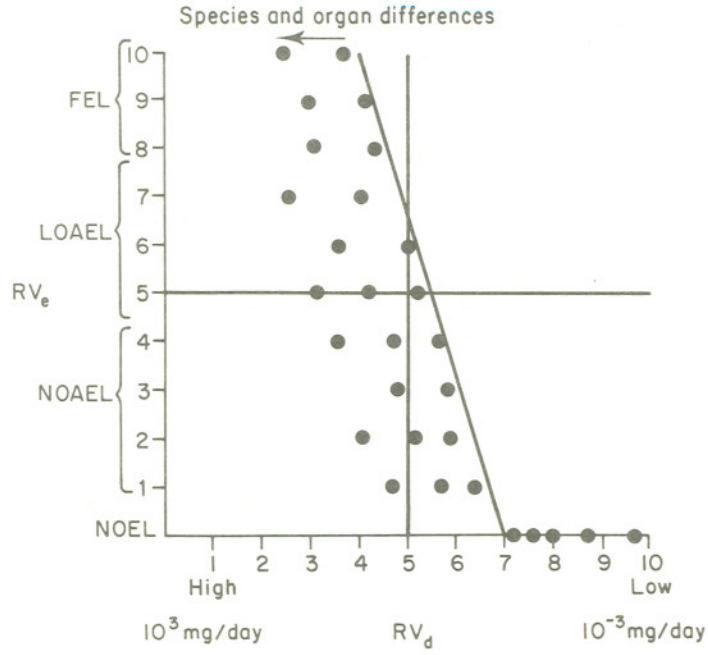

Figure 5 Hypothetical NOELs, NOAELs, LOAELs and FELs. $RV_d = 10$ if log MED < -3. $RV_d = -1.5 \log$ MED $+ 5.5$ if $-3 \leqslant \log$ MED $\leqslant 3$. $RV_d = 1$ if log MED > 3. From Durkin and Colman (1983)

Figure 5. Substances having an effect at a low dose will have a high rating on this scale.

Nonetheless, the variation in organ sensitivities is an important factor in defining the MED associated with a given dose level for a particular toxicant. This is illustrated in Figure 5, where hypothetical data in which NOELs, NOAELs, LOAELs, and FELs are plotted on a RV_d versus RV_e diagram. At low doses (i.e. high RV_d) no effects are observed. As the dose increases (i.e. the RV_d decreases), effect ratings become increasingly high. However, at a given effect level, multiple effects can be expected as the dose increases. The point furthest to the right represents the most sensitive organ or species, or the highest incidence of the effect. Consider the line drawn to the right of the effect level points that intersects the *x*-axis to the left of the NOELs. The slope of this line represents the maximum increase in the severity of effect with increasing dose and may be termed the apparent severity slope.

The application of the methodology has suggested that the RV_d versus RV_e plot may be useful to other Agency needs in the estimation of the NOELs or NOAELs used in the derivation of ADIs. Kushner *et al.* (1983) recently reviewed

the use of ADIs in Superfund implementation, and Stara and coworkers (Dourson and Stara, 1983; Stara *et al.*, 1980) have reviewed more general problems with the estimation and application of ADIs in the general regulatory process. Two problems involving the use and derivation of ADIs may be at least partially alleviated by using the RV_d versus RV_e plot. First, the Agency frequently has been criticized for not using all of the available data in estimating the NOAEL from which ADIs are derived. Using the RV_d versus RV_e plot along with an appropriate statistical method for estimating the apparent severity slope and the *x*-intercept (i.e. maximum NOEL), all of the available subchronic and chronic data could be used. Second, examples have been encountered where no suitable NOAELs or LOAELs are available from which an ADI could be derived. In such cases, data on FELs and NOELs could be used to estimate either the maximum NOEL or a suitable NOAEL (e.g. dose associated with an RV_e of 1 or 2). Again, this would be dependent on the development of an appropriate mathematical model to estimate the severity slope and *x*-intercept.

In addition to these immediate applications, it may be desirable to consider expanding the RV_d versus RV_e plot to include axes for duration of exposure, species, and incidence of response. This, however, would be a very complex undertaking that will await further development and applications of the basic RV_d versus RV_e plot.

7 SUMMARY AND CONCLUSION

This brief overview of current methodological developments cannot fully reflect the extent and complexity of the efforts required for these tasks. Some of the new developments related to both individual systemic toxicants and chemical mixtures, such as an improved approach to interspecies dose conversion, have been in progress for several years. Others, such as methodologies for partial lifetime health risk evaluation, the effects-ranking scheme and the determination of sensitive population subgroups, are relatively recent. Much effort is needed, both in the area of improved risk assessment methodology and in the area of toxicology and validation of the theoretical approaches. It is hoped that overviews such as this paper will stimulate the necessary research so that improved data and mechanistic theories can increase the reliability and accuracy of predictive toxicology. The USEPA is making every reasonable effort to improve its risk assessment approach and will continue to do so.

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