APPENDIX A

BACKGROUND INFORMATION ON THE ASSESSMENT OF
ADDITIVITY AND INTERACTIONS

A.1.   INTRODUCTION

The approaches to assessing the joint action of components of a mixture are based in large measure on the conceptual groundwork laid by Bliss (1939) and Finney (1971), and are mathematical rather than biological in nature. The approaches commonly known as dose addition and response addition, discussed in the following sections, are non-interactive forms of joint action that assume the chemicals in the mixture do not affect the toxicity of one another, i.e., that they act independently. These assumptions are the bases for methods of risk and health assessment discussed in the Guidance Manual. In addition, the assessment of interactions depends on being able to define what constitutes non-interaction.

The available studies of toxicological interactions often pose a problem for the health assessor because the results may be ambiguous, often due to poor study design, or the results of several studies on the same mixture may appear to be conflicting, or the relevance of the study or studies to the exposure scenario of interest is uncertain. Approaches for dealing with these uncertainties are introduced in this appendix and further discussed in Appendices B and C.

A.2.   MODELS FOR JOINT ACTION

A.2.1. DOSE ADDITION

As introduced in the Guidance Manual, dose addition, also known as concentration addition, simple similar action, and similar joint action, assumes that the components of a mixture behave as concentrations or dilutions of one another, differing only in their potencies (Bliss 1939; Finney 1971). The dose-response curves are parallel (i.e., the regression lines of probits on log doses are parallel), and tolerance (or susceptibility) to the components is completely positively correlated (the organisms most susceptible to chemical A also will be most susceptible to chemical B). The response to the mixture can be predicted by summing the doses of the components after adjusting for the differences in potencies. Dose addition is considered most appropriate for mixtures with components that affect the same endpoint by the same mechanism of action EPA (1986, 1990, 2000). It has been suggested that the requirement for parallel dose-response curves and complete correlation of tolerances may be too stringent (e.g., Plackett

and Hewlett 1952; Svendsgaard and Hertzberg 1994), and that in the low-dose region in which the response is linear, dose additivity may hold for independently-acting chemicals as well (Svendsgaard and Hertzberg 1994). Dose addition is the underlying assumption of the hazard index method and the toxic equivalency factor (TEF) approach (Sections 2.3.1 and 2.3.3).

The regression lines for two chemicals (1 and 2) that act in a dose additive manner can be represented as:

$$Y_1 = \beta \log x + \alpha_1 \tag{1}$$

$$Y_2 = \beta \log x + \alpha_2 \tag{2}$$

where x is dose or concentration, Y_i is the probit response for the ith chemical, β is the slope (by definition the same for both chemicals), and α_i is the intercept on the exposure axis (the value of Y when x is zero) for the ith chemical. The potency ρ of chemical 2 relative to chemical 1 is:

$$\log \rho = \frac{(\alpha_2 - \alpha_1)}{\beta} \tag{3}$$

Using equation 3 to convert the dose of the second chemical into an equivalent amount of the first, equation 2 can be rewritten as:

$$Y_2 = \beta \log(\rho \cdot x) + \alpha_1 \tag{4}$$

Thus, for a mixture of chemicals 1 and 2 in which the exposures are x_1 and x_2, the response is dose additive if it equals that produced by a dose $(x_1 + \rho x_2)$ of the first chemical alone, as expressed by the following equation:

$$Y = \alpha_1 + \beta \log(x_1 + \rho \cdot x_2) \tag{5}$$

Alternatively, if the mixture is regarded as a total dose x, in which the proportions of the two chemicals are π_1 and π_2, equation 5 can be written as:

$$Y = \alpha_1 + \beta \log(\pi_1 + \rho \pi_2) + \beta \log x \tag{6}$$

Equations 5 and 6 can be generalized for a greater number of components.

Relationships that may be useful in analyzing interactions data (Finney 1971) can be derived from equation 6. If for a mixture of defined proportions of chemical 1 and 2, some uniform measure of toxicity (risk-specific dose or equally effective dose, e.g., ED_{50}) is known for the two chemicals and designated by ζ_1 and ζ_2, respectively, then:

$$\zeta_2 = \frac{\zeta_1}{\rho} \tag{7}$$

The toxicity ζ_m of any mixture of chemicals 1 and 2 can be predicted as follows under the assumption of dose addition:

$$\zeta_m = \frac{\zeta_1}{(\pi_1 + \rho\pi_2)} \tag{8}$$

Equation 8 can also be written in the following form:

$$\frac{1}{\zeta_m} = \left(\frac{1}{\zeta_1}\right)\pi_1 + \left(\frac{\rho}{\zeta_1}\right)\pi_2 \tag{9}$$

Based on equation 7, $1/\zeta_2$ can be substituted for ρ/ζ_1 in equation 9 to give:

$$\frac{1}{\zeta_m} = \frac{\pi_1}{\zeta_1} + \frac{\pi_2}{\zeta_2} \tag{10}$$

This form of the equation can be used to predict the ED_{50} (or other uniform measure of toxicity) of a mixture from the proportions and ED_{50}s of the components.

A.2.2. APPLICATIONS OF DOSE ADDITION TO HEALTH AND RISK ASSESSMENT

The toxic equivalency (TEQ) approach and hazard index approach are based on the assumption of dose addition. The response to the mixture is considered dose additive if it equals that produced by a dose of the first chemical alone. The mixture dose (X), expressed as an equivalent dose of the first chemical alone, is:

$$X = \rho_1 x_1 + \rho_2 x_2 + \rho_3 x_3 + \cdots + \rho_n x_n \tag{11}$$

where ρ_i is the potency of the ith component relative to the first chemical and x_i is the concentration or dose of the ith component. Note that $\rho_1 = 1$, the potency of chemical 1 relative to itself.

In the TEQ approach, the first or index chemical is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which is assigned a toxic equivalency factor (TEF) of unity, representing its potency relative to itself. TEFs for the other active congeners are based on their potency relative to 2,3,7,8-TCDD. The concentrations or doses of all active congeners are multiplied by their TEF values and summed to give the *TEQs* for the mixture, which is the concentration of the mixture expressed as an equivalent concentration of the index chemical, 2,3,7,8-TCDD:

$$TEQs = TEF_1 C_1 + TEF_2 C_2 + TEF_3 C_3 + \cdots + TEF_n C_n = \sum_{i=1}^{n} TEF_i C_i \qquad (12)$$

where TEF_i is the potency of the ith component relative to 2,3,7,8-TCDD and C_i is the concentration of the ith component (ATSDR 1998; EPA 1994; Van den Berg et al. 1998). Equation 12 is equivalent to equation 5 of the Guidance Manual. The relative potency method for polycyclic aromatic hydrocarbons (PAHs) (ATSDR 1995; EPA 1993) is a similar application of dose addition. Additional information and references are provided in Section 2.3.4 of the Guidance Manual.

The hazard index approach uses 1/DL (where DL is a defined level of exposure such as an MRL or RfD) as an indicator of potency (because the larger the DL, the less the potency) for the components of a mixture. If E is the total mixture dose or exposure expressed as the equivalent dose of chemical 1, where chemical 1 can be any component of the mixture, then, under dose addition:

$$E = \frac{DL_1}{DL_1} E_1 + \frac{DL_1}{DL_2} E_2 + \frac{DL_1}{DL_3} E_3 + \cdots + \frac{DL_1}{DL_n} E_n \qquad (13)$$

where DL_i is the defined level for the ith component, and E_i is the exposure to the ith component, in the same units. Factoring out DL_1 from the numerators, equation 13 becomes:

$$E = DL_1 \left(\frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \frac{E_3}{DL_3} + \cdots + \frac{E_n}{DL_n} \right) \qquad (14)$$

Dividing both sides of equation 14 by DL_1 gives the expression for the hazard index (HI):

$$\frac{E}{DL_1} = HI = \frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \frac{E_3}{DL_3} + \cdots + \frac{E_n}{DL_n}$$

(15)

The hazard index approach is discussed further in Section 2.3.1 of the Guidance Manual.

Limitations of the hazard index approach include the requirement imposed by the dose addition model that the mode of action of the chemicals be similar, and the weakness of the assumption that the defined levels (MRLs or RfDs) represent isoeffective doses. Potential improvements to the approach include the use of toxicity thresholds or effective dose levels (e.g., $ED_{10}s$), rather than MRLs or other defined levels, but there are analytical problems in determining these values as well, and they are not available for most chemicals. Svendsgaard and Hertzberg (1994) have discussed the statistical issues associated with the hazard index approach.

A.2.3. RESPONSE ADDITION

Response addition, as introduced in the Guidance Manual (Section 2.3), *Response Addition*, also known as simple independent action and independent joint action (Bliss 1939), assumes that the chemicals act independently and by different modes of action. Because the modes of action are different, tolerance (or susceptibility) to the components is not necessarily positively correlated under response addition. The response to the mixture can be predicted from the responses to the components and the correlation of tolerances. Response addition is the underlying assumption of an approach to cancer risk assessment for mixtures and ACGIH's approach to assessing the hazard of occupational exposure to agents that act independently (Sections 2.3.5 and 3.1).

The form of response addition will be different depending on the correlation of susceptibility to the components of the mixture. If the organisms most sensitive to chemical 1 are also most sensitive to chemical 2, susceptibilities to chemicals 1 and 2 are completely and positively correlated. The correlation coefficient r is equal to one. The expected response P to the mixture of chemicals 1 and 2 at doses that individually produce responses P_1 and P_2 is equivalent to that for the chemical with the highest response. Thus:

$$P = P_1 \quad if \ r = 1 \quad and \quad P_1 > P_2$$
$$P = P_2 \quad if \ r = 1 \quad and \quad P_2 > P_1$$

(16)

In other words, if the dose of chemical 1 would be expected to cause a response in 8% of the animals and chemical 2 would be expected to cause a response in 17% of the animals, the expected response to the mixture of these two chemicals at these doses is 17% when susceptibilities are completely positively correlated.

If the organisms most sensitive to chemical 1 are least sensitive to chemical 2 and vice versa, susceptibilities to chemicals 1 and 2 are completely and negatively correlated. Under this circumstance, the predicted response to the mixture would be simply additive ($8 + 17 = 25\%$) as long as the total of the responses to chemicals 1 and 2 was less than unity.

$$P = P_1 + P_2 \quad \textit{if } r = -1 \quad \textit{and} \quad (P_1 + P_2) \leq 1 \tag{17}$$

Intermediate to these two extremes is the circumstance when the susceptibility to the two chemicals are statistically independent. In this case, some of the organisms that would not respond to chemical 1 would respond to chemical 2, so that the total response rate for the mixture is:

$$\begin{aligned} P &= P_1 + P_2(1 - P_1) \\ &= P_1 + P_2 - P_1 P_2 \end{aligned} \tag{18}$$

Using the same response rates as in the previous examples, the response to the mixture would be estimated as $100(0.08 + 0.17 - (0.08 \cdot 0.17)) = 23.6\%$.

The above equations can be generalized for a greater number of components.

A.2.4. APPLICATIONS OF RESPONSE ADDITION TO HEALTH OR RISK ASSESSMENT

The relationships of the equations for the various forms of response addition to their applications in risk assessment are more intuitively obvious than is the relationship of the equations for dose addition to such applications as the hazard index. Accordingly, the applications will not be discussed in detail here, but rather mentioned with a reference to the section of the Guidance Manual in which they are presented.

An approach similar to response addition assuming completely positive correlation of tolerances (equation 16 of this appendix) has been applied by ACGIH to the assessment of mixtures whose components are expected to cause effects that are independent from each other, such as purely local effects on different organ systems. The threshold limit for the mixture is considered to be exceeded only if the hazard quotient for at least one of the components exceeds unity (Section 3.1).

The calculation of total cancer risk (Section 2.3.5) is based on response addition with completely negative correlation of tolerances. The responses (risks) for the individual components of the mixture are summed to estimate the response to the mixture as in equation 17 of this appendix.

A.3. INTERACTIONS

A.3.1. INTRODUCTION TO INTERACTION MODELS

The assessment of interactions involves assumptions regarding what constitutes an additive or non-interactive response. Thus, the assumed form of additivity often drives experimental design and the assessment of joint action. Knowledge of the mode of action of the individual components of the mixture is often used in selecting a plausible additivity model.

If interactions appear to exist, as determined from deviations from the assumed form of additivity, mathematic models for quantifying the interactions may be used. Finney (1942, 1971) proposed the following interaction model, which is a modification of equation 5 for dose addition:

$$Y = \alpha_1 + \beta \log(x_1 + \rho \cdot x_2) + \kappa(\rho \cdot x_1 \cdot x_2)^{0.5} \tag{19}$$

where κ is the interaction coefficient. Positive values of κ indicate synergism, negative values indicate antagonism, and a value of zero indicates dose addition.

A.3.2. EXPERIMENTAL STUDIES

Experimental studies of toxicological interactions, particularly those designed primarily to investigate the mechanism of action of the chemical of interest, may not reflect the models discussed above. From the material already presented in this appendix, it follows that, in general, an understanding of the joint action of the components of a mixture depends upon an understanding of the dose-response relationships for the individual components. There are exceptions to this generalization. An example is the case where one component is known to be inactive with regard to the effect of concern. In this case, only the dose-response curves for the active component with and without the addition of the inactive component may be necessary.

Other interaction studies do use dose addition or response addition models in the evaluation of additivity versus interactions. For example, Smyth et al. (1969) used equation 10 to predict the toxicity (LD_{50}) of

the 350 possible binary mixtures of 27 industrial chemicals administered in equivolume combinations. (One pair of chemicals proved impossible because it reacted vigorously upon mixing before administration.) The ratio between the predicted (P) and observed (O) values, calculated for each pair, ranged from 0.23 to 5.09, indicating that the magnitude of deviation from dose additivity was approximately a factor of 5 or less. This is not a remarkable deviation from additivity and thus suggests that dose additivity is a reasonable default model for joint action. The upper end of the range of the deviation from additivity of 5 also has been used as the basis for a default "magnitude of interaction" factor in the modified WOE method (EPA 2000) described in Appendix B. Smyth et al. (1970) retested 53 chemical pairs from this set in equitoxic combinations. Because the distribution of ratios for the first (equivolume) study was skewed, the investigators normalized the ratios in that study and in the equitoxic study using the following adjustment:

> where *P/O>1*; adjusted ratio = *(P/O) – 1*
> where *P/O<1*; adjusted ratio = *1 – (O/P)*

With the adjusted ratios, a positive value indicates greater-than-additive joint action, a negative value indicates less-than-additive joint action, and a value of zero indicates additivity.

The equivolume and equitoxic experiments used different proportions of the chemicals for each pair. The difference in proportions should not affect the ability of equation 10 to predict the LD_{50} for the mixture. A comparison of the adjusted ratios in the equivolume and equitoxic experiments on the same pairs of chemicals showed that the correlation between the two sets of ratios was good. These results further support dose addition as a reasonable default model for joint action.

Further guidance regarding the evaluation of studies of joint toxic action is provided in ATSDR (2001).

A.3.3. ASSESSING THE RELEVANCE OF INTERACTIONS STUDIES TO HUMAN HEALTH

Much of the information available on toxicological interactions is for binary mixtures of chemicals. Most of the studies summarized in MIXTOX (EPA 1990), a database that focuses primarily on interactions relevant to noncancer toxicity, are for short durations of dosing. A large proportion of the studies in this database used a sequential rather than simultaneous exposure protocol, and the great majority focused on lethality or liver toxicity as an endpoint. When two or more studies were available on a particular binary mixture, the results were sometimes conflicting and the experimental variables different. Interpretation of this information is problematic when the objective is to predict the potential impact on public health from exposure to a mixture consisting of more than two chemicals, where exposure to these chemicals is occurring simultaneously, for extended durations, and at relatively low doses. Similar conclusions as to the relevance of the available interactions data to human health have been reached by Krishnan and Brodeur (1991) in their monumental review of interactions studies on both noncarcinogenic and carcinogenic endpoints.

Methods for predicting joint toxic action from this type of data include the Weight-of-Evidence (WOE) methods (EPA 2000; Mumtaz and Durkin 1992; Mumtaz et al. 1994) discussed in Appendix B and the Integral Search System (ISS) (DiCarlo and Woo 1994; Woo et al. 1994) discussed in Appendix C. The WOE methods require a careful evaluation of the available interactions data, supplemented by the evaluation of mechanistic, pharmacokinetic, and toxicological data, plus a consideration of structure activity relationships—for all binary combinations of chemicals in a mixture of concern. This degree of analysis may pose a problem in terms of the numbers of chemical pairs that would be of interest for mixtures associated with hazardous waste sites.

Potential solutions to this problem are likely to involve computer programs that perform the analyses automatically. One solution, offered by ISS, is to count, for each pair of chemicals, one "hit" if one (or more) studies have reported an interaction in an interaction category scored by that program, sum the hits in each category for all possible pairs, and compute a composite score for the mixture, weighted for the estimated importance of a given interaction category (such as synergism). A chemical pair with 6 studies showing synergism, 0 for promotion, 0 for antagonism, and 1 for inhibition would have a score of 1 for synergism, 0 for promotion, 0 for antagonism, and 1 for inhibition. The ISS also takes into account the potential interactions for a chemical without data by assessing the interactions of the structural or functional class to which the chemical belongs. It then uses the numbers of hits along with a weighting factor to calculate a "weighting ratio" that reflects the potential impact of interactions on the hazard of the mixture. The limitations of ISS are discussed in Appendix C.

Another potential solution is to develop ways to count each result in each interaction category (synergism, additivity, antagonism) for each pair of chemicals, and assess the variance of results and the statistical significance of the observed pattern. This method, developed by Durkin et al. (1995), based on the data in MIXTOX, can be used to assess the patterns of interactions between single chemicals, a chemical and a class, or between classes of chemicals. In addition, it can be used to define a class of chemicals based on empirical similarities, i.e., a class consisting of chemicals that appear to interact in a similar manner with one or more other chemicals. Significant interaction patterns for classes of chemicals could be used as "rules" for chemicals in those classes that lack interactions data, in support of WOE assessments. A limitation of this study was the paucity and variability of the interactions studies on any given pair of chemicals (data used for these analyses were current through 1991). Given the increased interest in the toxicological interactions of environmental contaminants, it is possible that considerably more data may be available now to support the patterns approach, making further development worthwhile.

A.4.   REFERENCES

ATSDR. 1995. Toxicological profile for polycyclic aromatic hydrocarbons (PAHs) (update). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

ATSDR. 1998. Toxicological profile for chlorinated dibenzo-*p*-dioxins (December 1998 final). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

ATSDR. 2001. Guidance for the preparation of an interaction profile. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Bliss CI. 1939. The toxicity of poisons applied jointly. Ann Appl Biol 26:585-615.

DiCarlo FJ, Woo Y. 1994. Ranking the carcinogenic potential of chemical mixtures: The Integral Search system and its use in evaluating hazardous waste sites. Drug Metab Rev 26:685-715.

Durkin P, Hertzberg R, Stiteler W, et al. 1995. The identification and testing of interaction patterns. Toxicol Lett 79:251-269.

EPA. 1986. U.S. Environmental Protection Agency. Guidelines for the health risk assessment of chemical mixtures. Fed Reg 51:34014-34025.

EPA. 1990. Technical support document on health risk assessment of chemical mixtures. U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/8-90/064.

EPA. 1993. Provisional guidance for quantitative risk assessment of polycyclic aromatic hydrocarbons. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment. NTIS PB94-116571.

EPA. 1994. Estimating exposures and risks. In: Estimating exposure to dioxin-like compounds. Volume III: Site-specific assessment procedures. External Review Draft. Washington, DC: Office of Research and Development. EPA/600/6-88/005C, 2-1 to 2-6.

EPA. 1999. Guidance for conducting health risk assessment of chemical mixtures (External scientific peer review draft). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. NCEA-C-0148.

Finney DJ. 1942. The analysis of toxicity test on mixtures of poisons. Ann Appl Biol 29:82-94.

Finney DJ. 1971. Probit analysis. 3rd ed. Cambridge University Press, 232-238, 246-247, 252-254, 262.

Krishnan K, Brodeur J. 1991. Toxicological consequences of combine exposures to environmental pollutants. Arch Complex Environ Studies 3(3):1,35.

Mumtaz MM, Durkin PR. 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicol Ind Health 8:377-406.

Mumtaz MM, De Rosa CT, Durkin PR. 1994. Approaches and challenges in risk assessments of chemical mixtures. In: Yang RSH, ed. Toxicology of chemical mixtures: Case studies, mechanisms and novel approaches. New York, NY: Academic Press, 565-597.

Plackett RL, Hewlett PS. 1952. Quantal responses to mixtures of poisons. J Royal Stat Soc B 14:141-163.

Smyth HF, Weil CS, West JS, et al. 1969. An exploration of joint toxic action: Twenty-seven industrial chemicals intubated in rats in all possible pairs. Toxicol Appl Pharmacol 14:340-347.

Smyth HF, Weil CS, West JS, et al. 1970. An exploration of joint toxic action. II. Equitoxic versus equivolume mixtures. Toxicol Appl Pharmacol 17:489-503.

Svendsgaard DJ, Hertzberg RC. 1994. Statistical methods for the toxicological evaluation of the additivity assumption as used in the environmental protection agency chemical mixture risk assessment guidelines. In: RSH Yang, ed. Toxicology of chemical mixtures. New York, NY: Academic Press, 100-155.

Van den Berg M, Birnbaum L, Bosveld ATC, et al. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 106(12):775-792.

Woo Y, Di Carlo FJ, Arcos JC, et al. 1994. Assessment of carcinogenic hazard of chemical mixtures through analysis of binary chemical interaction data. Environ Health Perspect 102(Suppl 9):113-118.

APPENDIX B

WEIGHT-OF-EVIDENCE METHODS

B.1 INTRODUCTION

The weight-of-evidence (WOE) methods for the assessment of chemical interactions described in this appendix were designed to facilitate the use of interactions data in the components-based assessment of noncancer health effects from exposure to chemical mixtures. As noted above, the hazard index method does not incorporate information on interactions among components of the mixture. A WOE method proposed by Mumtaz and Durkin (1992) was the first systematic attempt to address this need. The method implemented and expanded on the suggestion of the NRC (1989) that an uncertainty factor be used to account for interactions among components of a mixture. The value of the uncertainty factor can reflect the concern for interactions, and is modified using data regarding the WOE for interactions (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). As suggested by the NRC, the uncertainty factor is applied to the additivity-based hazard index to estimate an interactions-adjusted hazard index. Subsequent experience with the algorithm that is used to generate the interactions-adjusted hazard index has revealed, however, that it does not handle changes in the proportions of mixture components in a reasonable manner. The method remains useful in the qualitative prediction of whether hazard may be greater or less than indicated by the hazard index (Sections B.1.2 and B.2.2).

A modification to the WOE method was developed (ERG and Durkin 1995; EPA 2000) in order to explicitly incorporate information on the magnitudes of the pairwise interactions into the risk assessment. This modified method addresses some of the limitations of the original method, but introduces a new set of limitations: greater judgment may be required in the scoring of the weight-of-evidence and information on the magnitude of interactions is rarely available.

An abbreviated description of the original method was presented in the guidance manual; some of the information will be repeated here for the sake of completeness and to facilitate comparison of the two methods. The following sections provide additional details of these methods.

B.2   ORIGINAL WOE METHOD

B.2.1. BINARY WEIGHT OF EVIDENCE SCORES

The first step in applying the WOE method is to assess data relevant to joint action for each possible pair of chemicals in the mixture in order to make a qualitative binary weight-of-evidence (BINWOE) determination for interactions. The BINWOE determination is a classification that reflects the quality of the available information and categorizes the most plausible nature of the potential influence of one chemical on the toxicity of another chemical for a given exposure scenario (duration, route, and sequence). This determination includes evaluating information regarding the toxicity, pharmacokinetics, and mechanism of action of the individual chemicals; interactions data on each chemical pair; and interactions and mechanistic data on related chemicals. Although the earlier publications of the WOE method did not discuss the need for BINWOE determinations to take into account target organ (Durkin 1995; Mumtaz and Durkin 1992), experience in application of the WOE method has indicated that the WOE evaluations should be target-organ specific (Mumtaz et al. 1998). Two BINWOE determinations are made for each pair: one for the effect of chemical A on the toxicity of chemical B, and the other for the effect of chemical B on the toxicity of chemical A (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). The criteria and scoring system for the BINWOE determinations are presented in Table B-1.

The classification of direction of interaction in Table B-1 has the following categories: additive, greater-than-additive, less-than-additive, and indeterminate. The additive category refers to results that are additive by a defined model of additivity (e.g., dose or response addition), and results which demonstrate no effect of one chemical on the toxicity of the other. The greater-than-additive category refers to synergism or potentiation. The less-than-additive category refers to antagonism, inhibition, or masking. Indeterminate refers to instances of ambiguous, conflicting, or no data.

The classification of the quality of the data in Table B-1 includes two main categories: mechanistic understanding and toxicological significance. The rating for mechanistic understanding reflects the quality of the available mechanistic data supporting a toxicological interaction and the extent to which this information indicates the direction of the interaction. Mechanistic information is information regarding the manner in which a chemical causes a given toxic effect or interaction, and may include chemical, biological, and physical processes at the molecular level and at higher levels of biological or physiological organization. The rating for toxicological significance reflects the quality of the available toxicological interactions data and the extent to which it indicates that the chemicals will interact in a manner that significantly impacts the health of the exposed population. Both the mechanistic and

toxicological categories allow for, and encourage, the use of structure-activity data in reaching conclusions. The "modifiers" in Table B-1 are used when the mechanistic and toxicological ratings do not account for the additional concerns for differences in duration, sequence, bioassay (*in vitro* versus *in vivo*), or route of exposure between the site-specific exposures and the mechanistic and toxicological data used for the BINWOE determinations (Mumtaz and Durkin 1992).

The qualitative direction and alphanumeric data quality terms are shown in the left column of Table B-1. The corresponding direction factor and numerical data quality weighting factors are shown in the right column. The qualitative scores can be converted to a single numerical score by multiplying the direction factors (labeled "Direction" in the table) and the data quality weighting factors (labeled "Weight" in the table). Thus, an alphanumeric (qualitative) BINWOE classification of >II.B.2.a.i corresponds to greater-than-additive interaction, mechanistic data on related chemicals, inferred toxicological significance, different duration or sequence, *in vivo* data, and anticipated route of exposure. The corresponding numerical BINWOE score is $+1(0.71)(0.71)(0.79)(1)(1) = +0.40$.

The data quality weighting factors were selected using the following reasoning: the optimum score for data quality is unity, and corresponds to the first level of scoring (categories I and A for the primary classifications of mechanistic or toxicological significance and 1, a, and I for the modifiers). For the primary classifications, the value of 0.71 was selected for the second level of scoring (categories II and B) so that if both factors were selected the score would be about one-half of the optimum score ($0.71 \cdot 0.71 \approx 0.50$). Similarly, for the third level of scoring (categories III and C), the value of 0.32 was selected so that if both factors were selected the score would be about one-tenth of the optimum score ($0.32 \cdot 0.32 \approx 0.1$). For the modifiers, a value of 0.79 was selected for the second level of scoring (2, b, and ii) so that all three factors combined would lower the score by a factor of about 0.5 ($0.79 \cdot 0.79 \cdot 0.79 \approx 0.5$). The numerical weighting values reflect judgment as to the relative importance of the data quality classifications in determining the weight of evidence (Durkin 1995).

The BINWOE determinations do not explicitly consider the relevance of dose to the anticipated exposure scenario. It is not uncommon to find that, for a well-studied binary mixture, the available information suggests that no interactions occur at low doses, but that an interaction, either greater-than-additive or less-than-additive, occurs at higher doses. The BINWOE for this situation would reflect the interaction observed at higher doses. Dose *is* taken into account in the calculation of interaction factors (Section B.2.2). Additional guidance for the determination of BINWOEs is provided in the ATSDR (2001) *Guidance for the Preparation of an Interaction Profile*.

Table B-1. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

Classification		Factor
Direction of Interaction		**Direction**
=	Additive	0
>	Greater than additive	+1
<	Less than additive	−1
?	Indeterminate	0
Quality of the Data		**Weighting**
Mechanistic Understanding		
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.	1.0
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur have not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.	0.71
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.	0.32
Toxicological Significance		
A.	The toxicological significance of the interaction has been directly demonstrated.	1.0
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.	0.71
C.	The toxicological significance of the interaction is unclear.	0.32
Modifiers		
1.	Anticipated exposure duration and sequence.	1.0
2.	Different exposure duration or sequence.	0.79
a.	*In vivo* data	1.0
b.	*In vitro* data	0.79
i.	Anticipated route of exposure	1.0
ii.	Different route of exposure	0.79

Weighting Factor = Product of Weighting Scores: Maximum = 1.0, Minimum = 0.05

BINWOE = Direction Factor x Weighting Factor: Ranges from −1 through 0 to +1

*Adapted from Mumtaz and Durkin (1992) and Mumtaz et al. (1994a)

B.2.2.   QUALITATIVE WOE METHOD

A qualitative WOE approach, focusing on application of the BINWOE scores to hazardous waste-site assessment, was suggested by Mumtaz and Durkin (1992). This approach is appropriate for a mixture where the scaled doses (hazard quotients) for all the components are similar, or toxicologically significant. The qualitative BINWOE scores for the components, if similar in direction, are the basis for a conclusion. For example, consider a mixture of four components, all present at toxicologically significant levels. The number of possible chemical pairs in a mixture of N components is $(N^2-N)/2$. Thus, this mixture of 4 components has 6 pairs of components and potentially 12 BINWOEs. Suppose nine of the BINWOEs are greater-than-additive (positive) with alphanumeric classifications indicating a relatively high degree of confidence, and the remaining three BINWOEs are additive (0), also with relatively high degrees of confidence. In this case, the weight of evidence suggests that the mixture is likely to pose a greater hazard than that indicated by the hazard index.

A likely pattern of qualitative BINWOEs for a mixture is a mixed pattern (some greater than additive, some less than additive, and some additive BINWOEs). In this case, the qualitative WOE approach is extended to include conversion of the qualitative BINWOE scores to numerical scores, and summing the scores to give a combined score. If the combined BINWOE score is positive and significantly different from zero, then the weight of evidence suggests that the mixture is likely to pose a greater hazard than indicated by the hazard index. Conversely, if the combined BINWOE score is negative and significantly different from zero, then the weight of evidence suggests that the health hazard is unlikely to be greater than indicated by the hazard index. Professional judgment is used in the interpretation of the impact of the WOE on the hazard index.

Although the above WOE method was developed for assessing interactions for noncarcinogenic effects, the qualitative WOE method is equally applicable to assessing interactions for carcinogenic effects.

B.2.3.   INTERACTION FACTORS

The quantitative application of the WOE method is described in this section, and continues through Section B.2.5. As mentioned previously, this quantitative application does not handle changes in the proportions of mixture components in a reasonable manner, and is no longer in use. The description is retained in this document because the method represents an interesting and original attempt to modify the hazard index for interactions.

In this quantitative application, the BINWOEs are used as interaction terms in the calculation of interaction factors, $IF_{i,j}$ and $IF_{j,i}$ (where $IF_{i,j}$ is the effect of j on the toxicity of i and $IF_{j,i}$ is the effect of i on the toxicity of j) as follows:

$$IF_{i,j} = \frac{HQ_i}{HI_{add}} \cdot BINWOE_{i,j} \ (HQ_i \cdot HQ_j)^{0.5} \tag{1}$$

$$IF_{j,i} = \frac{HQ_j}{HI_{add}} \cdot BINWOE_{j,i} \ (HQ_i \cdot HQ_j)^{0.5} \tag{2}$$

The two equations are identical except that equation 1 calculates the interaction factor for the effect of j on the toxicity of i, and equation 2 calculates the interaction factor for the effect of i on the toxicity of j.

The first set of terms in these equations weights the interaction factor by the contribution of the chemical whose toxicity is affected to the total toxicity of the mixture, expressed as the ratio of the hazard quotient (HQ_i) of that chemical to the total additivity-based hazard index (HI_{add}) of the mixture (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). This approach is adapted from one developed by Durkin (1981) to account for asymmetrical interactions under the assumption of dose additivity. Asymmetrical interactions are those in which the magnitude and sometimes the direction of the interaction vary with the proportions of the components in the mixture. It should be noted that there is a slight difference between the algorithms in Mumtaz and Durkin (1992) and Mumtaz et al. (1994a). In the 1994 paper (Eq. 2a and 2b), the term $HQ_i/(HQ_i+HQ_j)$ is used. In the 1994 review (Mumtaz et al. 1994a), the term HQ_i/HI_{add} is used.

The BINWOE score is the interaction term, which quantifies concern with interaction for a chemical pair. Estimation of the BINWOE score was discussed in the previous section.

The last set of terms in these two equations is the geometric mean of the hazard quotients for the two chemicals. Finney (1942, 1971) proposed a similar term for modeling symmetrical interactions under the assumption of dose additivity. The use of the geometric mean lowers the value of the interaction factor as exposure to either of the two chemicals falls below the defined level (denominator of the hazard quotient, e.g., MRL) for that chemical, i.e., as either hazard quotient falls below unity. This property of the WOE approach is consistent with the general observation that as exposure levels and the probability of responses due to the individual components decrease, the toxicological significance of interactions in a mixture will decrease (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). In addition, the use of the geometric mean lowers the value of the interactions factor as the hazard quotients of the two components deviate from each other. This is consistent with the assumption that the greatest departure from additivity

(greatest interaction) will occur when both components of a binary mixture are present in equitoxic amounts. This assumption also is expressed in Finney's model of a deviation from dose additivity (Finney 1942, 1971), presented in Appendix A (Section A.3.1).

B.2.4.   WOE

The next step in this method is to sum the interaction factors to express the overall direction and weight of evidence for the toxicological interactions of the site-specific mixture, WOE_S.

$$WOE_S = \sum_{i \neq j} \sum IF_{i,j} \tag{3}$$

The double summation sign indicates that each component of the mixture is evaluated for the effect that every other component could have on its toxicity. The overall process (substituting the full expression for the interaction factors into equation 3) can be represented by equation 4.

$$WOE_S = \sum_{i \neq j} \sum \frac{HQ_i}{HI_{add}} \cdot BINWOE_{i,j} \cdot (HQ_i \cdot HQ_j)^{0.5} \tag{4}$$

The WOE_S score has no absolute or clear interpretation. For example, a score of -0.16 could be a composite of interaction factors for antagonism (-0.223) and synergism (+0.060) or a composite of interaction factors all of which reflect very low confidence in antagonism (e.g., -0.01, -0.04, -0.05, -0.01, -0.02, -0.03). Therefore, Mumtaz and Durkin (1992) recommended that the WOE be normalized by dividing the WOE_S by the maximum possible score that the site-specific mixture would have generated if all the interactions information had indicated a consistent direction of interaction and had been assigned weighting scores indicating the highest possible degree of confidence (BINWOE determinations of I.A.1.a.i with corresponding BINWOE scores of 1.0). Because the BINWOE scores are 1, they essentially drop out of equations 1 and 2 for the interactions factors, and therefore out of equation 4. Accordingly, the maximum possible score, WOE_{MAX}, can be calculated by summing the simplified expressions for the interaction factors as follows:

$$WOE_{MAX} = \sum_{i \neq j} \sum \frac{HQ_i}{HI_{add}} \cdot (HQ_i \cdot HQ_j)^{0.5} \tag{5}$$

The normalized WOE for the site-specific mixture, WOE_N, is:

$$WOE_N = \frac{WOE_S}{WOE_{MAX}} \tag{6}$$

The WOE_N is an expression of the strength of the evidence suggesting that interactions may be toxicologically significant relative to the highest possible level of confidence that can be expressed for the site-specific mixture using this method. For example, consider the previously mentioned site-specific mixture with an estimated WOE_S of -0.16 (the sum of interaction factors indicating less-than-additive and greater-than-additive interactions). Suppose the WOE_{MAX} for this site is 0.75. The WOE_N is calculated as $-0.16/0.75 = -0.21$. Thus, the strength of the available data on the binary interactions, when used with the exposure data from the site, suggests that the net effect of interactions for the mixture is likely to be less-than-additive, as indicated by the minus sign in the WOE_S and WOE_N scores. Relative to (hypothetical) interactions data of the highest possible quality for the same mixture and exposures, overall confidence in the assessment of less-than-additive toxicity for this site-specific mixture is about 20%, as indicated by the magnitude of the WOE_N score (Mumtaz and Durkin 1992; Mumtaz et al. 1994a).

B.2.5. INTERACTIONS-BASED HAZARD INDEX

Consistent with the suggestion by the NRC (1989) that the hazard index be adjusted for interactions through the application of an uncertainty factor, and with EPA and ATSDR approaches to assessing the noncancer toxicity of individual chemicals, Mumtaz and Durkin (1992) suggest that the hazard index be adjusted for the uncertainty of interactions by the application of an uncertainty factor. The uncertainty factor is modified by the normalized WOE score, WOE_N. The adjustment is performed as follows:

$$HI_I = HI_{add} \times UF_I^{WOE_N} \tag{7}$$

where HI_I is the interactions-based hazard index, HI_{add} is the additivity-based hazard index, and UF_I is an uncertainty factor for interactions. Thus, the hazard index is multiplied by the uncertainty factor for interactions to the power of WOE_N.

The NRC (1989) discussed the use of an uncertainty factor in the range of 1 to 100 depending on the available interactions information and the concentrations of the components. Mumtaz and Durkin (1992) note that the value of the uncertainty factor UF_I could be set by taking into account the concern for the magnitude of an interaction, but that suitable data regarding magnitude generally are not available. For the purposes of illustration, an uncertainty factor of 10 has been used in the various examples and exercises performed with this WOE methodology. Because WOE_N can range from -1 (for the highest possible confidence in less-than-additive interactions) to +1 (for the highest possible confidence in greater-than-additive interactions), UF_I to the power of WOE_N can range from 0.1 to 10. The net effect can be to increase *or decrease* the hazard index by a factor of 10. The WOE approach therefore differs from the NRC (1989) approach, which uses an uncertainty factor only to increase the hazard index. It

also differs from ATSDR and EPA approaches to assessing the noncancer toxicity of individual chemicals through the derivation of MRLs, RfDs, and RfCs, in which uncertainty factors are applied to make the health criterion more conservative.

As an example of the application of the WOE method, the WOE_N of -0.21 discussed in the previous section and an additivity-based hazard index of 2 are substituted into equation 7 to estimate the interactions-based hazard index, as follows:

$$HI_I = 2 \cdot 10^{-0.22} = 1.2 \tag{8}$$

For a WOE_N of +0.22, and a hazard index of 2, the interactions-based hazard index would be 3.3. A larger value of WOE_N, +0.75, applied to a hazard index of 2 would result in an interactions-based hazard index of 11.

B.2.6. STRENGTHS AND LIMITATIONS OF THE ORIGINAL WOE METHOD

The highly prescriptive method for BINWOE classification is designed to encourage a consistent application of the methodology. The application was considered consistent by expert toxicologists who reviewed the results of exercises in which 5-6 teams of toxicologists and risk assessors independently determined BINWOE classifications for the same pairs of chemicals, using the same data (Mumtaz et al. 1994b).

The separation of mechanistic understanding from toxicological significance and equal weighting of the these two categories has been questioned on the grounds that mechanistic understanding is important in risk assessment only as it serves to support or modify toxicological significance. Based on analyses of interactions data, the sequence of exposure appears to have a more profound impact on the nature of the interaction than does route or possibly duration (Hertzberg and Durkin 1994). It has been suggested that the sequence of exposure be separated from duration and given a separate weighting factor to better reflect the impact of sequence on the nature of the interaction (Durkin 1995).

The algorithms do not provide a means for using information on the magnitudes of the interactions for specific pairs of components, should such information be available. Rather, the magnitudes of the interactions among the components of a mixture are represented by a single uncertainty factor, which is modified by the WOE determinations, and then applied to the hazard index. Given the scarcity of suitable data for determining the magnitude of interactions, this may not be a limitation. The normalization process was considered useful as an indicator of confidence in the assessment of direction of interactions

for the site-specific mixture and when there is a need to compare scores across hazardous waste sites. It also constrained the value of the interactions-modified uncertainty factor within reasonable limits (0.1 to 10).

The WOE method (Mumtaz and Durkin 1992; Mumtaz et al. 1994a) has undergone evaluation, and appeared to perform well qualitatively, and quantitatively under some circumstances. The application of the method for deriving BINWOE classifications was considered consistent by expert toxicologists who reviewed the results of exercises in which several teams of toxicologists and risk assessors independently determined BINWOE classifications for the same pairs of chemicals (Mumtaz et al. 1994b). In tests of the WOE method to predict the toxicity of some simple chemical mixtures to animals, BINWOEs for three pairs of chemicals qualitatively predicted whether the results of animal studies would be less-than-additive, additive, or greater-than-additive (Mumtaz et al. 1998). Used with an exponential dose-response model and dose addition to model relative kidney weights, the quantitative WOE method closely predicted the observed dose-response in female rats for intermediate-duration oral exposure to a mixture of four nephrotoxic chemicals with similar modes of action (Mumtaz et al. 1998). The observed dose-response was less than dose additive. The BINWOEs were focused on renal toxicity, and the uncertainty factor used in the algorithm was 10. The WOE method underestimated the relative liver weights in the same animals. The observed dose-response for relative liver weight was slightly greater than dose additive. Thus, the WOE method did not predict toxicity to a target organ that was different from the one for which the BINWOEs were derived. The WOE method slightly overpredicted the observed dose-response for relative kidney weight in male rats for a mixture of dissimilarly acting nephrotoxins (in female rats, the data variability was so great that the exponential model did not fit the *observed* responses) (Mumtaz et al. 1998). Although these results are suggestive, limitations of this test of the complete WOE method include the substantial variability in the responses of individual animals, small numbers of animals per group, testing of only two dose levels of the mixtures, and lack of rationale for using relative organ weight as an index of toxicity (several other indicators of renal and hepatic toxicity were monitored in the studies that provided the experimental data [Jonker et al. 1993, 1996]).

Subsequent experience with the WOE method revealed, however, that the algorithm does not handle changes in proportions of mixture components in a reasonable manner. Therefore, ATSDR has discontinued the use of the algorithm and will use a qualitative WOE approach (Section B.2.2), as suggested by Mumtaz and Durkin (1992), until an appropriate algorithm can be developed or selected, and fully evaluated. The WOE algorithm and other approaches of this type must be tested to ensure that they behave in a reasonable and consistent manner with regard to the underlying assumptions and that their predictions are reasonable representations of experimental or known exposure outcomes.

B.3. MODIFIED WOE METHOD

B.3.1. MODIFIED BINARY WEIGHT OF EVIDENCE SCORES

The modified WOE method, proposed by ERG and Durkin (1995), further developed by EPA, and adopted as part of EPA (2000) mixtures guidance, employs an alternative weight-of-evidence classification scheme that focuses on a more integrated interpretation of the data. The suggested numerical weights for the various classifications range from 0 to 1.0 as in the original methodology. As in the original method, two BINWOE determinations are made for each pair: one for the effect of chemical A on the toxicity of chemical B, and the other for the effect of chemical B on the toxicity of chemical A. Unlike the original methodology, less weight is given to less-than-additive interactions under circumstances where there is some uncertainty regarding the interaction (categories II and III). The scheme is shown in Table B-2.

This modified scheme facilitates the integration of toxicological and mechanistic data to support classification in an appropriate category. In common with the original scheme, it encourages the use of structure-activity information to support a classification. Because it is less prescriptive than the original BINWOE classification scheme, the modified scheme may require a greater degree of judgment in actual use.

Like the original method, the modified method does not take dose into account during the BINWOE determination, but rather during application of the algorithms (Section B.3.2).

B.3.2. MODIFIED INTERACTIONS-BASED HAZARD INDEX

The modified WOE method modifies each component's hazard quotient (where HQ_i is the hazard quotient of the ith component) by the influences of all the other potentially interacting components, resulting in a hazard quotient modified for interactions (HQ_{i_I}). The interactions-modified hazard quotients are then summed to estimate the interactions-based hazard index (HI_I):

$$HQ_{i_I} = \sum_{i \neq j}^{n} HQ_i \, f_{j,i} \, M_{i,j}^{BINWOE_{i,j}\theta_{i,j}} \qquad (9)$$

$$HI_I = \sum_{i=1}^{n} HQ_{i_I} \qquad (10)$$

The overall process is shown in the following equation (EPA 2000). Some of the terms in equations 9-11 are modified slightly from those in the cited publications for consistency with the terms used in the original methodology.

$$HI_I = \sum_{i=1}^{n} (HQ_i \cdot \sum_{j \neq i}^{n} f_{j,i} \, M_{i,j}^{BINWOE_{i,j}\theta_{i,j}}) \qquad (11)$$

Table B-2. Modified Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

Default Weighting Factors

Category	Description	Direction Greater than additive	Less than additive
I.	The interaction has been shown to be relevant to human health effects and the direction of the interaction is unequivocal.	1.0	-1.0
II.	The direction of the interaction has been demonstrated *in vivo* in an appropriate animal model and the relevance to potential human health effects is likely.	0.75	-0.50
III.	An interaction in a particular direction is plausible but the evidence supporting the interaction and its relevance to human health effects is weak.	0.5	0.0
IV.	The assumption of additivity has been demonstrated or is accepted because the information is: A. Insufficient to determine the direction of any potential interaction. B. Insufficient to determine whether any interaction would occur. C. Adequate as evidence that no toxicologic interaction between the components is plausible.	0.0	0.0

*Adapted from EPA 2000

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The term $f_{j,i}$ scales the interactions contribution of chemical j by its importance relative to all the other chemicals interacting with chemical i. The toxicological importance is represented by the hazard quotient:

$$f_{j,i} = \frac{HQ_j}{HI_{add} - HQ_i} \tag{12}$$

$M_{i,j}$ is the magnitude of the interaction, defined as an estimate of the maximum effect that chemical j has on the threshold or risk-specific dose (e.g., ED_{10}) of chemical i. When, as is often the case, data regarding the magnitude are not available, a default value of 5 is used, which is consistent with the upper end of the range of deviation from additivity shown by Smyth et al. (1969). The direction of the interaction is not incorporated into M, but rather is part of the term $BINWOE_{i,j}$, which is the BINWOE score. Positive values indicate the interaction is greater-than-additive, negative values indicate less-than-additive, and the value of zero indicates additivity. $M_{i,j}$, raised to the power of $BINWOE_{i,j} \bullet \theta_{i,j}$, functions as an uncertainty or modifying factor in the estimation of the interactions-based hazard quotients. The term $\theta_{i,j}$ reflects the degree to which components i and j are present in equitoxic amounts, based on the hazard quotients. This term is incorporated into the algorithm to account for the assumption that the greatest deviation from additivity will occur when both components in a binary mixture are present in equitoxic amounts (EPA2000) . As discussed previously, this assumption is explicit in a model of a deviation from dose additivity proposed by Finney (1942, 1971). The measure of the deviation from equitoxic amounts is the ratio ($\theta_{i,j}$) of the geometric mean to the arithmetic mean of the hazard quotients:

$$\theta_{i,j} = \frac{(HQ_i \cdot HQ_j)^{0.5}}{(HQ_i + HQ_j)/2} \tag{13}$$

As HQ_i approaches HQ_j, $\theta_{i,j}$ approaches 1, and as HQ_i and HQ_j deviate from each other, $\theta_{i,j}$ approaches 0. Thus, the term $\theta_{i,j}$ reflects how close to equitoxic are the two chemicals' doses. The value for of $\theta_{i,j}$ is the same (0.94) for two components with hazard quotients of 0.01 and 0.02, or 0.1 and 0.2, or 1 and 2.

B.3.3. STRENGTHS AND LIMITATIONS OF THE MODIFIED WOE METHOD

The modified WOE method may require more judgment in the determination of BINWOEs than does the original WOE method. The increased flexibility and the integration of toxicological and mechanistic information could lead to a more holistic assessment, but the flexibility also could lead to an erratic application of the methodology. Consistency of application has not been tested.

Although both WOE methods use BINWOE scores to modify an uncertainty (or magnitude) factor that can be based on the magnitude of the interactions, the original method focuses on a single uncertainty factor for the entire mixture, whereas the modified method focuses on individual magnitude factors (M) for the effect of each component on the toxicity of each other component. Thus, the potential advantage of the modified WOE method is that information on the magnitude of interactions can be applied directly to the hazard quotient of the chemical whose toxicity is affected. A default magnitude value of 5 is used when data regarding magnitude are not available. This method is relatively new, and, as of this writing, has not been tested to determine whether toxicologists can apply it consistently and how well it predicts the toxicity of simple mixtures. It does appear to handle changing proportions of mixture components in a reasonable manner.

B.4. PRACTICAL CONSIDERATIONS FOR IMPLEMENTATION OF A WOE METHOD IN PUBLIC HEALTH ASSESSMENTS

The number of possible pairs in a mixture of N components is $(N^2-N)/2$. Thus a mixture of 4 chemicals has 6 possible pairs needing 12 BINWOEs, a mixture of 6 chemicals has 15 possible pairs needing 30 BINWOEs, and a mixture of 9 chemicals has $(81-9)/2 = 36$ possible pairs needing 72 BINWOEs. Obviously, the practicality of either WOE method may be an issue for mixtures with more than 4–5 components because of the large numbers of BINWOE determinations that would be required. If an algorithm is used, the calculations are fairly extensive.

Some ways of addressing this issue of practicality are as follows:

- Limit the use of the WOE method to those situations where clarification of the public health hazard is needed, such as sites where exposures to individual components are high enough, relative to health guidelines, that additivity and interactions may result in a significant health hazard.
- Focus the BINWOE effort on chemical pairs that frequently pose the above situation for ATSDR health assessments.
- Make BINWOE determinations available through an easily accessible and readily updated medium, such as the ATSDR website or Interaction Profiles.
- Further develop the patterns approach to analyzing and predicting interactions (Durkin et al., 1995) (see also Appendix A, Section A.3.3) as a potentially cost-effective means of generating BINWOEs.

- Develop a spreadsheet programmed with the appropriate equations to carry out the WOE calculations (if an appropriate algorithm is developed/fully evaluated/selected).

B.5.   REFERENCES

ATSDR. 2001. Guidance for the preparation of an interaction profile. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Durkin PR. 1981. An approach to the analysis of toxicant interactions in the aquatic environment. In: Branson DR, Dickson KL, eds. Aquatic toxicology and hazardous assessment. 4th Conference, ASTM STP 737. American Society for Testing and Materials, 388-401.

Durkin P. 1995. Development of mixtures assessment methods: Guidelines for application of the binary weight-of-evidence methodology. Submitted to The Kevric Company, Inc., Silver Spring, MD. SERA TR 95-018-001a.

Durkin P, Hertzberg R, Stiteler W, et al. 1995. The identification and testing of interaction patterns. Toxicol Lett 79:251-264.

EPA. 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. EPA/630R-00/002.

ERG and Durkin, P. 1995. A weight-of-evidence approach for mixtures risk assessment. Prepared by Syracuse Environmental Research Associates, Inc. and Eastern Research Group, Inc. under ERG Task No. 8495-61, as part of EPA Contract No. 68-C1-0030 for National Center for Environmental Assessment, U.S. EPA, Cincinnati, OH.

Finney DJ. 1942. The analysis of toxicity test on mixtures of poisons. Ann Appl Biol 29:82-94.

Finney DJ. 1971. Probit analysis. 3rd ed. Cambridge University Press, 262 (equation 11.83).

Jonker D, Woutersen RA, van Bladeren PJ, et al. 1993. Subacute (4-wk) oral toxicity of a combination of four nephrotoxins in rats: Comparison with the toxicity of the individual compounds. Food Chem Toxicol 31(2):125-136.

Jonker D, Woutersen RA, Feron VJ. 1996. Toxicity of mixtures of nephrotoxicants with similar or dissimilar mode of action. Food Chem Toxicol 34:1075-1082.

Hertzberg RC, Durkin PR. 1994. Influence of exposure timing and other factors on toxicologic interaction patterns. Conference on Temporal Aspects in Risk Assessment for Noncancer Endpoints, Dayton, OH, April 18-20. Registration and Abstract Booklet, 65.

Mumtaz MM, Durkin PR. 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicol Ind Health 8:377-406.

Mumtaz MM, De Rosa CT, Durkin PR. 1994a. Approaches and challenges in risk assessments of chemical mixtures. In: Yang RSH, ed. Toxicology of chemical mixtures: Case studies, mechanisms and novel approaches. New York, NY: Academic Press, 565-597.

Mumtaz MM, Durkin PR, Diamond GL, et al. 1994b. Exercises in the use of weight-of-evidence approach for chemical-mixture interactions. In: Andrews JS, Frumkin H, Johnson BL, et al., eds. Hazardous Waste and Public Health: International Congress on the Health Effects of Hazardous Waste, May 3-6, 1993, Atlanta, GA. Princeton, NJ: Princeton Scientific Publishing Co., Inc., 637-642.

Mumtaz MM, De Rosa CT, Groten J, et al. 1998. Estimation of toxicity of chemical mixtures through modeling of chemical interactions. Environ Health Perspect 106(Suppl 6):1353-1360.

NRC. 1989. Mixtures. In: Drinking water and health. Vol. 9. National Academy of Sciences, National Research Council, Safe Drinking Water Committee. Washington, DC: National Academy Press, 93–107, 121-132, 168-170.

Smyth HF, Weil CS, West JS, et al. 1969. An exploration of joint toxic action: Twenty-seven industrial chemicals intubated in rats in all possible pairs. Toxicol Appl Pharmacol 14:340-347.

APPENDIX C

THE INTEGRAL SEARCH SYSTEM FOR RANKING HAZARDS OF
MIXTURES OF CARCINOGENS

C.1. INTRODUCTION

The Integral Search System (ISS) was designed to facilitate the use of interactions data in the component-based assessment of carcinogenic effects from exposure to chemical mixtures (DiCarlo and Woo 1994; Woo et al. 1994). An overview of this method was presented in the Guidance Manual (Section 2.3.6); some of that information will be repeated here as needed for understanding of the complete method, the details of which are presented in the following sections. The method also has been reviewed by Mumtaz et al. (1994) and EPA (2000).

Like the weight-of-evidence (WOE) methods (Appendix B), the ISS uses data for binary mixtures to predict the hazard of exposure to mixtures of three or more chemicals. The ISS is a software package that integrates three EPA and National Cancer Institute databases on binary interactions of carcinogens with other carcinogens (Arcos et al. 1988), with promoters (Rao et al. 1989) and inhibitors (Bagheri et al. 1988-89). A user's manual provides directions for using the software (Polansky and Woo 1994). The ISS calculates a weighting ratio that reflects the ratio of greater-than-additive to less-than-additive interactions for the components of a mixture. In addition, ISS can be used to estimate a level of concern based on the slope factors (potencies) of the components and the weighting ratio. Because the estimate of level of concern does not include a consideration of exposure level, its usefulness is limited.

C.2. WEIGHTING RATIO

The ISS computer program generates a list of all possible binary combinations of the mixture components. It then searches for interactions data for each pair and each category of interaction (synergism [syn], promotion [pro], antagonism [ant], and inhibition [inh]). A "name pair hit" (H_A) is tallied when information on a pair of components is located for any of these interaction categories. For each pair of components, the program registers only the first hit encountered for each interaction category. The total count of name pair hits for all component pairs is designated by, for example, $H_{A\,syn}$ for synergism.

For each pair with no name pair hits, the ISS searches for interactions between members of the structural or functional classes to which the components lacking data belong. Hits identified in this manner are called "class pair hits." The total number of class pair hits for each category of interaction is statistically adjusted in order to take into account the frequency and distribution of different interaction categories and the representativeness of the classes in ISS, and to insure that the inferred value will not exceed the value of a name pair hit, which is unity. The derivation of this adjustment procedure is highly complex, requiring eight pages of explanation in the software manual (Polansky and Woo 1994). The result is an "inferred class pair value" (H_B).

The name pair hits (H_A) and inferred class pair values (H_B) for each interaction category are then totaled as shown in the following example for synergism:

$$H_{syn} = H_{A\ syn} + H_{B\ syn} \tag{1}$$

The extent of hazard modification due to interactions among mixture components is estimated as a weighting ratio (*WR*):

$$WR = \frac{1 + (pH_{syn} + qH_{pro})}{1 + (rH_{ant} + sH_{inh})} \tag{2}$$

where p, q, r, and s are weighting factors for the effectiveness of the four types of interactions to modify the hazard of the mixture based on additivity. Based on their review of the interactions literature, Woo et al. (1994) consider the following to be reasonable default values: $p = 0.3$, $q = 0.7$, $r = 0.3$, and $s = 0.6$. These default values have been incorporated into the ISS program, but can be changed by the user.

The presence of the number one in both the numerator and denominator of the weighting ratio prevents the weighting ratio from reducing to zero when both H_{syn} and H_{pro} are zero, or from becoming infinity when both H_{ant} and H_{inh} are zero. When no interaction information is available or when the information for greater-than-additive interactions is equal to that for less-than-additive interactions, the weighting ratio is unity, and the hazard assessment is unchanged.

C.3.   INHERENT CANCER HAZARD AND LEVEL OF CONCERN

Calculation of the inherent hazard, like the calculation of total cancer risk discussed in the Guidance Manual (Section 2.3.5), is based on the assumption of response additivity with a completely negative correlation of tolerances. The ISS program, however, does not include exposure concentration or dose as

part of the procedure. Instead, ISS calculates the inherent hazard as the sum of the cancer slope factors of the components, expressed in units of $(mmole/kg\text{-}day)^{-1}$. The sum is then converted by ISS to an exponent index, which is a linear scale of hazard indicators that approximately parallels the ranking of exponents of the slope factors (Table C-1).

Table C-1. Correspondence Among Slope Factors, Exponent Indexes, and Concern Levels*

Slope Factor $(mmole/kg/day)^{-1}$	Exponent Index	Concern Level
0 to $<5x10^{-5}$	0 to <1	Low
$5x10^{-5}$ to $<5x10^{-1}$	1 to <4	Marginal
$5x10^{-1}$ to $<5x10^{0}$	4 to <6	Low–moderate
$5x10^{0}$ to $<5x10^{1}$	6 to <8	Moderate
$5x10^{1}$ to $<5x10^{2}$	8 to <10	High–moderate
$5x10^{2}$ to $\approx 5x10^{7}$	10 to ≈ 14	High

*Adapted from DiCarlo and Woo (1994)

This correspondence table was developed for a set of 134 chemicals with known slope factors. The correspondence table constitutes an interface with structure-activity relationship (SAR) analysis, which is being used to provide a judgment regarding carcinogenic potential and a rough estimate of slope factor (as concern level) for data-poor chemicals through the computer program OncoLogic (DiCarlo and Woo 1994; Polansky and Woo 1994; Woo et al. 1995).

ISS multiplies the inherent hazard, in units of exponent index, by the weighting ratio (from Section C.2). The resulting weighted exponent index is then converted by ISS to a weighted total slope factor and to a corresponding concern level, ranging from low to high, as shown in the right column of Table C-1.

C.4.   STRENGTHS AND LIMITATIONS

The obvious strengths of the ISS are that it performs the analyses automatically, and can be applied to mixtures with relatively large numbers of components, including components not presently included in the database, provided those components can be assigned to an appropriate class of chemicals within the database. The ISS does not, however, evaluate the relevance of the data to the anticipated exposure

scenario in the manner that the WOE method does. Nor does it provide an indication of the strength of the evidence for a particular interaction. A serious limitation of the ISS is that exposure levels are not taken into account during the procedure. As discussed in the Guidance Manual (Section 2.3.6), this limitation may be circumvented, at least in part, by restricting the use of this method to components whose exposures fall within a limited range of estimated risks or are considered toxicologically significant. The weighting ratio could then be used as an alternative weight-of-evidence score for interactions. Another serious limitation is that the class-class interaction ratings for pairs of chemicals with no data tend to dominate the score. ISS and OncoLogic are in use by EPA, but both are undergoing further review and development, which may address the limitation regarding the class-class interactions.

C.5.   REFERENCES

Arcos JC, Woo Y, Lai DY. 1988. Database on binary combination effects of chemical carcinogens. Environ Carcin Rev (J Environ Sci Health) C6(1):v-xiv.

Bagheri D, Doeltz MK, Fay JR, et al. 1988-89. Database of inhibitors of carcinogenesis. Environ Carcin Rev (J Environ Sci Health) C6(3):vii-xviii.

DiCarlo FJ, Woo Y. 1994. Ranking the carcinogenic potential of chemical mixtures: The Integral Search system and its use in evaluating hazardous waste sites. Drug Metab Rev 26:685-715.

EPA. 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. EPA/630R-00/002.

Mumtaz MM, De Rosa CT, Durkin PR. 1994. Approaches and challenges in risk assessments of chemical mixtures. In: Yang RSH, ed. Toxicology of chemical mixtures: Case studies, mechanisms and novel approaches. New York, NY: Academic Press, 565-597.

Polansky G, Woo Y. 1994. The Integral Search system for cancer hazard ranking of complex chemical mixtures: Software user manual. Prepared by Sciences Applications International Corp., Falls Church, VA and U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC. SAIC Project No. 01-1027-03-4683-000, 4-5,12-22, 30-32.

Rao VR, Woo Y, Lai DY, et al. 1989. Database on promoters of chemical carcinogenesis. Environ Carcin Rev (J Environ Sci Health) C7(2):vii-xxxvi.

Woo Y, Di Carlo FJ, Arcos JC, et al. 1994. Assessment of carcinogenic hazard of chemical mixtures through analysis of binary chemical interaction data. Environ Health Perspect 102(Suppl 9):113-118.

Woo Y, Lai DY, Argus MF, et al. 1995. Development of structure-activity relationship rules for predicting carcinogenic potential of chemicals. Toxicol Lett 79:219-228.