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 this Framework 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 environmental scientists 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 this Framework, 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 end point by the same mechanism of action (EPA 1986, 1988, 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, the TEF approach for CDDs, and RPF approaches for carcinogenic effects from PAHs and neurological effects from groups of insecticides with common mechanisms (Section 3.3.5).

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

$$Y_1 = \beta \log x + \alpha_1 \quad (1)$$

$$Y_2 = \beta \log x + \alpha_2 \quad (2)$$

where x is dose or concentration, Y_i is the probit response for the i^{th} 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 i^{th} chemical. The potency ρ of chemical 2 relative to chemical 1 is:

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

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

$$Y_2 = \beta \log(\rho \bullet x) + \alpha_1 \quad (4)$$

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:

$$Y = \alpha_1 + \beta \log(x_1 + \rho \bullet x_2) \quad (5)$$

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:

$$Y = \alpha_1 + \beta \log(\pi_1 + \rho \pi_2) + \beta \log x \quad (6)$$

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 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:

$$X = \rho_1 x_1 + \rho_2 x_2 + \rho_3 x_3 + \cdots + \rho_n x_n \quad (11)$$

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

In the TEQ approach for CDDs and related compounds, the first or index chemical is 2,3,7,8-TCDD, which is assigned a TEF of unity, representing its potency relative to itself. TEFs for the other 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:

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

where TEF_i is the potency of the i^{th} component relative to 2,3,7,8-TCDD and C_i is the concentration of the i^{th} component (ATSDR 1998b; EPA 2010b; Van den Berg et al. 2006). Equation 12 is equivalent to Equation 5 of the Framework manual.

The relative potency method for PAHs (ATSDR 1995b; EPA 1993) is a similar application of dose addition. Additional information and references are provided in Section 3.3.5 of the Framework 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:

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

where DL_i is the defined level for the i^{th} component, and E_i is the exposure to the i^{th} component, in the same units.

Factoring out DL_1 from the numerators, Equation 13 becomes:

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

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

$$\frac{E}{DL_1} = HI = \frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \frac{E_3}{DL_3} + \cdots + \frac{E_n}{DL_n} \quad (15)$$

The hazard index approach is discussed in Section 3.3.2 of the Framework manual.

Limitations of the hazard index approach include the requirement imposed by the dose addition model that the MOA 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 BMD or effective dose levels (e.g., BMD_{10} or ED_{10} values), rather than MRLs or other defined levels. The EPA OPP's (EPA 2002b) approach for cumulative risk assessment of common effects from groups of pesticides sharing a common mechanism uses an RPF approach linked with a POD (e.g., BMD_{10})/MOE approach to characterizing the risk. See Sections 3.3.5 and 4.8 for more details on how this approach incorporates uncertainty factors into these assessments.

A.2.3. Response Addition

Response addition, as introduced in Section 3.3.1, also known as simple independent action and independent joint action (Bliss 1939), assumes that the chemicals act independently and by different MOAs. Because the MOAs are different, tolerance (or susceptibility) to the components is not necessarily positively correlated under response addition. The response to the mixture (expressed as the percent in a population responding) can be predicted from the responses to the individual components and the correlation of tolerance distributions (also termed susceptibility distributions) among components of the mixture (the proportion of members of a population responding as the exposure level of the component increases). Response addition is the underlying assumption of an approach to cancer risk assessment for mixtures at Superfund sites, EPA's and ATSDR's approach to noncancer risk assessment when exposure levels for components are near the individual NOAELs from well-designed toxicology

studies, and ACGIH's approach to assessing the hazards of occupational exposure to agents that act independently.

The form of response addition for populations will be different depending on the correlation of susceptibility to the components of the mixture. If the individuals 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:

$$\begin{aligned} P &= P_1 \quad if\ r=1 \quad P_1 > P_2 \\ P &= P_2 \quad if\ r=1 \quad P_2 > P_1 \end{aligned} \tag{16}$$

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

If the individuals 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 of the population 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.

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

Intermediate to these two extremes is the circumstance when the susceptibility to the two chemicals are statistically independent; the order of individuals showing toxic effects from chemical 1 has no apparent relationship with the ordering of individuals showing toxic effects from chemical 2 (r=0). 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:

$$\begin{aligned} P &= P_1 + P_2(1 - P_1) \\ &= P_1 + P_2 - P_1P_2 \end{aligned} \tag{18}$$

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 general form of the equation for multiple component mixtures is: $P_{mixture} = 1- (1-P_1) * (1-P_2) * (1-P_3) \ldots..$

The above equations can be generalized for a greater number of components. EPA (2000) commented that response addition formulas for populations, as illustrated in the examples above, have received limited use in risk assessment because detailed data for tolerance distributions are often not available and the concepts of tolerance correlation only works well if there are two chemicals in a mixture. Nevertheless, several applications of response addition assumptions are described in the next section.

A.2.4. Applications of Response Addition to Health or Risk Assessment

An approach similar to response addition assuming completely positive correlation of tolerances (Equation 16 of this appendix for a two-component mixture: $P_{mixture} = P_1$, if $r = 1$ and $P_1 > P_2$; $P_{mixture} = P_2$, if $r = 1$ and $P_2 > P_1$) 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 HQ for at least one of the components exceeds unity (Section C.1).

The calculation of total cancer risk based on response addition with completely negative correlation of tolerances has been recommended as an approach for adding cancer risks for mixtures of a few chemicals (EPA 2000; see Chapter 2). 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. EPA (2000) recommended that the full general equation for independently acting carcinogens [i.e., $P_{mixture} = 1- (1-P_1) * (1-P_2) * (1-P_3) \ldots..$] be applied to mixtures with more than a few carcinogens.

For low exposure levels (i.e., levels near individual component NOAELs from well-designed toxicology studies), toxicologically dissimilar chemicals are assumed to be independent, and response addition is assumed to be useful for noncancer risk assessment by EPA (2000) and this ATSDR framework (see Chapter 2). As such, when exposure to each component of a mixture is below the RfD, RfC, or MRL (estimated risk from each component = 0), the risk of adverse outcome from the mixture is usually assumed to be negligible. EPA (2000) noted that in these cases, "0 is used to denote a risk that is either subthreshold (a true zero risk) or small enough to be general considered virtually safe." EPA (2000) further noted that when the number of components in a mixture is large, and all component exposures are

close to, but below, respective guidance values (RfD, RfC, or MRL), “the toxicity data should be carefully examined to ensure that all effects and MOAs are being considered when deciding functional independence.” With poor quality data supporting the guidance value or the exposure assessment, “the conclusion of negligible risk is similarly uncertain (EPA 2000).”

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 MOA 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:

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

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. Early Experimental Studies Examining Dose Additivity

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. 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.

A.3.3. Evaluating Interaction Studies

To assess potential additivity and interactions that may occur among chemicals in a mixture and the effect that interactions will have on the inherent toxicity of the individual components of the mixture requires a thorough evaluation of the available studies on joint toxic action for the mixture and/or components of the mixture. The studies should be assessed based on the quality of the study and the applicability of the study design to predicting interactions or additivity.

ATSDR has adopted the NRC (1984) Guidelines for Assessing the Quality of Individual Studies, which appear in *Toxicity Testing: Strategies to Determine Needs and Priorities.* The NRC considers a report of scientific findings adequate for use in health hazard assessment if the report meets the following basic criteria:

1. All elements of exposure are clearly described.
2. Results in test subjects are predictive of human response, and test subjects are sensitive to the effects of the substance.
3. Controls are comparable with test subjects in all respects except the treatment variable.
4. End points answer the specific questions addressed in the study.
5. Observed effects are sufficient in number or degree to establish a dose-response relationship that can be used in estimating the hazard to the target species.
6. Both the design and the interpretation of the study allow for appropriate statistical analysis of the data.

Criteria for good studies further developed with the use of systematic reviews (Lynch et al. 2016; Rooney et al. 2014).

ATSDR recommends that good quality studies designed to assess the possible mode by which two or more chemicals affect a biological outcome should include:

1. Characterization of the effects of the individual components (and their dose-response relationships) on the outcome.
2. Generation of a hypothesis regarding the mode of joint action (e.g., dose addition or response addition).
3. Prediction of responses to mixtures of the components based on the postulated mode of joint action.
4. Observations of the response to mixtures of the components.
5. Statistical comparison of the predicted responses with the observed responses to the mixture.

These criteria are reflective of, and supplemented by, the criteria articulated by Borgert et al. (2001) for evaluating toxicological interaction studies:

1. Dose-response curves for the mixture components should be adequately characterized.

2. An appropriate “no-interaction” hypothesis should be explicitly stated and used as the basis for assessing synergy and antagonism.

3. Combination of mixture components should be assessed across a sufficient range (of exposure levels and mixing ratios) to support the goal of the study.

4. Formal statistical tests should be used to distinguish whether the response produced by a dose combination is different (larger or smaller) from that predicted by the ‘no-interactions” hypothesis (dose addition or response addition).

5. Interactions should be assessed at relevant levels of biological organizations.

An illustration of an adequate type of study involves two chemicals (A and B) that both individually affect a biological outcome. Dose-response data for each chemical alone indicate that linear dose-response models are adequate to describe dose-response relationships, and that A is 3 times more potent than B. Based on postulated joint additive action in which the null hypothesis is that the two chemicals behave as if they were concentrations or dilutions of one another (dose addition), a mixture of 1 dose unit of A plus 3 dose units B would be predicted to produce a response equivalent to that produced by:

- 2 dose units of A alone,
- 6 dose units of B alone, or
- a mixture of 0.33 dose unit of A and 5 dose units of B.

If observed responses to the mixture are greater than predicted responses, evidence is provided of a greater-than-dose-additive joint action. Conversely, if observed responses are less than predicted responses, there is evidence of a less-than-dose-additive joint action. If the dose-response relationships for the components and the mixture are not linear (e.g., show a sigmoidal shape), these specific predictions do not apply. With adequate characterization of the individual sigmoidal dose-response relationships, however, sufficient predictions of the combined effect by either dose addition or response addition can be calculated, and statistical tests comparing observed and predicted responses can be applied to assess deviations from either of these “no-interactions” hypotheses.

Unfortunately, the early toxicological literature on possible interactions among chemicals contains only limited numbers of studies that have all of the features of an optimal joint toxic action study. A standard design that was often followed (2x2 factorial design) involves a zero dose group (control), and chemicals A and B tested alone at doses of A1 and B1 and in combination at a dose of A1+B1. This type of design does not provide a full characterization of joint action, and the statistical analysis provided in such studies

often provides only information as to which treatment results are significantly different from other treatment results, rather than an indication of whether the results are indicative of a departure from dose addition or response addition (i.e., an interaction).

More complete discussion of statistical methods (and study design characteristics) to compare predicted and observed responses to mixtures are discussed by Berenbaum (1981), Bosgra et al. (2009), Calabrese (1991), Gennings et al. (2004, 2005), Hertzberg et al. (2013), Lutz et al. (2002), Scholze et al. (2014), and Svendsgaard and Hertzberg (1994).

Interaction studies also should be evaluated as to whether other components of the experimental design are relevant for assessing potential health outcomes of populations living near hazardous waste sites, including exposure route, duration of exposure, sequence of chemical administration, vehicle, dose and mixing ratio, and end points. Inhalation, oral, and dermal exposure are the most likely routes of exposure for human populations, and emphasis should be placed on interaction studies using these exposure routes. In the absence of data for a particular exposure route, data from other exposure routes may be used to predict interactions and health outcomes.

Use of data from another route would be based on the assumption that once a chemical has entered the body, there are no route-specific differences in toxicity or potency. However, this assumption may not be true if portal-of-entry effects or first-pass effects occur. First-pass effect refers to the metabolism that can take place in the portal-of-entry tissue, prior to entry into the systemic circulation, and can modulate the dose to remote or systemic target tissues in a route-dependent fashion. First-pass effect is usually considered with oral exposure because many chemicals are directly delivered from the gastrointestinal tract to the liver via the portal vein. The respiratory tract can also exhibit a first-pass effect after inhalation exposure. Although parenteral exposure is not an exposure route of concern, parenteral administration studies should be reviewed and evaluated if few or no studies using more relevant routes are available, because these data can provide valuable information on potential interactions and can provide mechanistic data. The relevance of parenteral studies to interactions involving oral exposure to the metals, however, needs careful consideration because parenteral administration bypasses homeostatic mechanisms and potential points of interaction related to absorption from the gastrointestinal tract.

Interactions among chemicals in a mixture can vary with duration of exposure. This is particularly true for chemicals that are toxic following chronic exposure but have low acute toxicity, or for chemicals whose biotransformation involves enzyme induction. When reviewing interaction data, the applicability

of the results to different exposure durations should be carefully considered. The toxicity/carcinogenicity and toxicokinetic databases for the chemicals of concern may provide useful information to support or refute extrapolation across exposure durations.

Interaction studies have utilized two patterns of administration: simultaneous and sequential. In the simultaneous administration study design, the mixture components are administered at the same time, or virtually the same time, using the same or different exposure routes. As this pattern of administration most closely resembles environmental exposure, greater emphasis should be placed on these data. Prior to 1991, many interaction studies employed a sequential pattern of administration, in which a chemical that alters metabolism or physiology in a known manner was administered before a single dose or exposure of the chemical of concern, in order to investigate the impact on the second chemical's toxicity (Hertzberg and Durkin 1994; Mumtaz and Durkin 1992). This study design provided data useful in elucidating the mechanism of action of the second chemical, but may not be as useful in understanding potential joint toxic action involving low-level, long-term simultaneous exposure.

Dose or exposure level and mixing ratio are also important factors to consider when evaluating interaction studies. In general, an understanding of the dose-response relationships for the individual components of the mixture is important for understanding potential interactions and health outcomes following exposure to the mixture. For example, if the dose tested is much lower than the threshold for the toxic end point of concern, then a potential interaction may not be detected by the study. On the other hand, if the dose used is too high, the dose may overwhelm the normal metabolic processes, resulting in different metabolites or an accumulation of a particular metabolite. Similarly, when examining potential interactions for a certain health effect, it is important to examine what other effects are occurring at the tested doses, and, in particular, whether the dose is so high that it is causing serious health effects in other organ systems, or death. Likewise, results from an interaction study evaluating a mixture with relative proportions of components different from the relative proportions in environmental mixtures may be of uncertain relevance to evaluating potential deviations from additivity in environmental mixtures.

A.4. REFERENCES

ATSDR. 1995b. Toxicological profile for polycyclic aromatic hydrocarbons. U.S. Department of Health and Human Services, Agency for Toxic Substances Disease Registry. http://www.atsdr.cdc.gov/ToxProfiles/tp69.pdf. July 8, 2015.

ATSDR. 1998b. Toxicological profile for chlorinated dibenzo-p-dioxins. U.S. Department of Health and Human Services, Agency for Toxic Substances Disease Registry. http://www.atsdr.cdc.gov/ToxProfiles/tp104.pdf. July 8, 2015.

Berenbaum MC. 1981. Criteria for analyzing interactions between biologically active agents. Adv Cancer Res 35:269-335.

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

Borgert CJ, Price B, Wells CS, et al. 2001. Evaluating chemical interaction studies for mixture risk assessment. Hum Ecol Risk Assess 7(2):259-306. 10.1080/20018091094376.

Bosgra S, van Eijkeren JC, Slob W. 2009. Dose addition and the isobole method as approaches for predicting the cumulative effect of non-interacting chemicals: A critical evaluation. Crit Rev Toxicol 39(5):418-426. 10.1080/10408440902787592.

Calabrese EJ. 1991. Pragmatic regulatory approaches for assessing complex mixtures of carcinogens. A. Comparative potency method. In: Multiple chemical interactions. Chelsea, MI: Lewis Publishers, 619-622.

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

EPA. 1988. Technical support document on health risk assessment of chemical mixtures. Washington, DC: U.S. Environmental Protection Agency. EPA600890064. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=435329. July 8, 2015.

EPA. 1993. Provisional guidance for quantitative risk assessment of polycyclic aromatic hydrocarbons. U.S. Environmental Protection Agency. EPA600R93089. PB94116571. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=466885. July 21, 2015.

EPA. 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. Washington, DC: U.S. Environmental Protection Agency. EPA630R00002. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=4486. July 2, 2015.

EPA. 2002b. Guidance on cumulative risk assessment of pesticide chemicals that have a common mechanism of toxicity. U.S. Environmental Protection Agency. http://www.epa.gov/oppfead1/trac/science/cumulative_guidance.pdf. May 8, 2015.

EPA. 2010b. Recommended Toxicity Equivalence Factors (TEFs) for human health risk assessments of 2,3,7,8-tetrachlorodibenzo-p-dioxin and dioxin-like compounds. U.S. Environmental Protection Agency. PB2011106152. EPA100R10005. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P1009HJ9.txt. November 19, 2016.

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

Finney DJ. 1971. Probit analysis. 3rd ed. London: Cambridge University Press.

Gennings C, Carter WH, Jr., Carchman RA, et al. 2005. A unifying concept for assessing toxicological interactions: changes in slope. Toxicol Sci 88(2):287-297. 10.1093/toxsci/kfi275.

Gennings C, Carter WH, Jr., Carney EW, et al. 2004. A novel flexible approach for evaluating fixed ratio mixtures of full and partial agonists. Toxicol Sci 80(1):134-150. 10.1093/toxsci/kfh134.

Hertzberg RC, Durkin PR. 1994. Influence of exposure timing and other factors on toxicologic interaction patterns. In: Conference on temporal aspects in risk assessment for noncancer endpoints, 18-20 April 1994, Dayton Ohio. Agency for Toxic Substances Disease Registry, U.S. Environmental Protection Agency, U.S. Army Research Detachment, 65.

Hertzberg RC, Pan Y, Li R, et al. 2013. A four-step approach to evaluate mixtures for consistency with dose addition. Toxicology 313(2-3):134-144. 10.1016/j.tox.2012.10.016.

Lutz WK, Vamvakas S, Kopp-Schneider A, et al. 2002. Deviation from additivity in mixture toxicity: Relevance of nonlinear dose-response relationships and cell line differences in genotoxicity assays with combinations of chemical mutagens and gamma-radiation. Environ Health Perspect 110 Suppl 6:915-918.

Lynch HN, Goodman JE, Tabony JA, Rhomberg LR. 2016. Systematic comparison of study quality criteria. Regulatory Toxicology and Pharmacology, 76: 187-198.

Mumtaz MM, Durkin PR. 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicol Ind Health 8(6):377-406.

NRC. 1984. Preface. In: Toxicity testing. Strategies to determine needs and priorities. Washington, DC: National Research Council, 61-62. http://www.nap.edu/catalog/317/toxicity-testing-strategies-to-determine-needs-and-priorities. December 2, 2015.

Plackett RL, Hewlett PS. 1952. Quantal responses to mixtures of poisons. J R Stat Soc Ser B 14(2):141-163.

Scholze M, Silva E, Kortenkamp A. 2014. Extending the applicability of the dose addition model to the assessment of chemical mixtures of partial agonists by using a novel toxic unit extrapolation method. PLoS ONE 9(2):e88808. 10.1371/journal.pone.0088808.

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 17:498-503.

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: Yang RSH, ed. Toxicology of chemical mixtures. Case studies, mechanisms, and novel approaches. San Diego, CA: Academic Press, 599-642.

Van den Berg M, Birnbaum LS, Denison M, et al. 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93(2):223-241. 10.1093/toxsci/kfl055.

APPENDIX B. CHEMICAL INTERACTIONS WEIGHT-OF-EVIDENCE (WOE) METHODS

B.1 INTRODUCTION

The 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 a 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 by 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: (1) greater judgment may be required in the scoring of the WOE; and (2) information on the magnitude of interactions is rarely available.

An abbreviated description of the original method was presented in the main body of the Mixtures 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. BINWOE 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 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 earlier publications of the WOE method did not discuss the need for target organ consideration in BINWOE determinations (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 interactions 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 that 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).

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

Sources: Mumtaz and Durkin 1992; Mumtaz et al. 1994a

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 WOE (Mumtaz and Durkin 1992).

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 *Guidance for the Preparation of an Interaction Profile* (ATSDR 2001).

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 (HQs) for all of 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 WOE 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 WOE 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 WOE 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:

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

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

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 HQ (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 of the interaction, and sometimes the direction of the interaction, vary with the proportions of the components in the mixture.

The BINWOE score is the interaction term that quantifies concern for interaction between 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 HQs 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 HQ; e.g., MRL) for that chemical (i.e., as either HQ 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 HQs 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.

B.2.4. WOE

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

$$WOE_S = \sum_{i \neq j}\sum IF_{i,j} \quad (3)$$

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.

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

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 of the interaction 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:

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

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

$$WOE_N = \frac{WOE_S}{WOE_{MAX}} \quad (6)$$

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:

$$HI_I = HI_{add} \times UF_I^{WOE_N} \quad (7)$$

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 1–100 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:

$$HI_I = 2 \cdot 10^{-0.22} = 1.2 \quad (8)$$

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 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 (Mumtaz and Durkin 1992).

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 (see Boobis et al. 2011), 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 (0.1–10).

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 MOAs (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 mixture component exposure levels in a reasonable manner. Hertzberg and Teuschler (2002) pointed out that for the conditions involving perfect evidence for synergy (when all $BINWOE_{ij} = 1$), the value of the equation describing the interaction-based hazard index ($HI_{int} = HI \times UF_I^{WOEN}$) becomes constant, regardless of changes in mixture composition. Hertzberg and Teuschler (2002) also noted that the uncertainty factor for interaction (UF_I) works differently than uncertainty factors for RfDs or MRL. Weak data lead to larger uncertainty factors for RfD/MRL development, thereby leading to lower, more public-health-protective, values of estimated safe dose; weak interaction data, in contrast, have minimal influence on hazard index values and thus, do not make the hazard index formula more or less public health protective. ATSDR does not recommend the use of the algorithm and recommends a qualitative WOE approach (Section B.2.2), as suggested by Mumtaz and Durkin (1992).

B.3. MODIFIED WOE METHOD

B.3.1. Modified BINWOE Scores

The modification of the original WOE method that was adopted as part of EPA's mixtures guidance (EPA 2000) employs an alternative WOE 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.

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

Default Weighting Factors			
		Direction	
Category	Description	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

Source: EPA 2000

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 HQ (where HQ_i is the HQ of the i^{th} component) by the influences of all the other potentially interacting components, resulting in a HQ modified for interactions (HQ_{i_I}). The interactions-modified HQs are then summed to estimate the interactions-based hazard index (HI_I):

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

$$HI_I = \sum_{i=1}^{n} HQ_{i_I} \tag{10}$$

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.

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

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

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

$M_{i,j}$ 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 that 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 HQs. The term $\theta_{i,j}$ reflects the degree to which components *i* and *j* are present in equitoxic amounts, based on the HQs. 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 (EPA 2000). 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 HQs:

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

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 the two chemicals' doses are. The value for of $\theta_{i,j}$ is the same (0.94) for two components with HQs 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 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 HQ of the chemical whose toxicity is affected. A default magnitude value of 5 is used when data regarding magnitude are not available.

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 >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. U.S. Department of Health and Human Services, Agency for Toxic Substances Disease Registry. http://www.atsdr.cdc.gov/interactionprofiles/interaction_profile_guidance.pdf. July 2, 2015.

Boobis A, Budinsky R, Collie S, et al. 2011. Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Crit Rev Toxicol 41(5):369-383. 10.3109/10408444.2010.543655.

Durkin PR. 1981. Approach to the analysis of toxicant interactions in the aquatic environment. In: Aquatic toxicology and hazard assessment: Fourth conference. American Society for Testing and Materials, 388-401.

Durkin P, Hertzberg R, Stiteler W, et al. 1995. The identification and testing of interaction patterns. Toxicol Lett 79(1-3):251-264.

EPA. 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. Washington, DC: U.S. Environmental Protection Agency. EPA630R00002. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=4486. July 2, 2015.

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

Finney DJ. 1971. Probit analysis. 3rd ed. Cambridge University Press,

Hertzberg RC, Durkin PR. 1994. Influence of exposure timing and other factors on toxicologic interaction patterns. In: Conference on temporal aspects in risk assessment for noncancer endpoints, 18-

20 April 1994, Dayton Ohio. Agency for Toxic Substances Disease Registry, U.S. Environmental Protection Agency, U.S. Army Research Detachment, 65.

Hertzberg RC, Teuschler LK. 2002. Evaluating quantitative formulas for dose-response assessment of chemical mixtures. Environ Health Perspect 110(Suppl 6):965-970.

Jonker D, Woutersen RA, Feron VJ. 1996. Toxicity of mixtures of neprotoxicants with similar or dissimilar mode of action. Food Chem Toxicol 34(11-12):1075-1082.

Jonker D, Woutersen RA, van BPJ, 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.

Mumtaz MM, Durkin PR. 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicol Ind Health 8(6):377-406.

Mumtaz MM, DeRosa CT, Durkin PR. 1994a. Approaches and challenges in risk assessment of chemical mixtures. In: Yang RSH, ed. Toxicology of chemical mixtures. Case studies, mechanisms, and novel approaches. San Diego, CA: Academic Press, 565-597.

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

Mumtaz MM, Durkin PM, Diamond GL, et al. 1994b. Exercises in the use of weight-of-evidence approach for chemical-mixture interactions. In: Hazardous waste and public health: International Congress on the health effects of hazardous waste. Princeton, NJ: Princeton Scientific Publishing Co., 637-642.

NRC. 1989. Drinking water and health. Vol. 9. Washington, DC: National Academy of Sciences, National Research Council, National Academy Press, Safe Drinking Water Committee, 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. METHODS USED OR PROPOSED BY OTHER AGENCIES

C.1. AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS (ACGIH)

ACGIH first discussed its procedure for dealing with exposure to mixtures in 1963 (ACGIH 1984); the procedures have changed little to the present day. ACGIH (2015) recommends additivity approaches for the assessment of occupational hazard to mixtures of chemicals. For mixtures of two or more hazardous agents that act on the same organ system, the ratio of the exposure concentration to the TLV for each component is summed (dose addition, hazard index approach). If the sum exceeds one, then the TLV for the mixture is considered as being exceeded. Exceptions to the hazard index approach can be made when there is good reason to expect that the chief effects of the components are not dose additive (i.e., are independent). According to ACGIH (2015), this can occur when components of the mixture do not have similar toxicological effects or target organs. When the effects are expected to be independent, the TLV for the mixture is exceeded only if at least one component has a HQ that exceeds unity. In effect, the hazard index for the mixture would be the highest HQ for any of the components. (This resembles response addition with completely positive correlation of tolerances, Appendix B.) ACGIH (2015) recommends evaluating synergism or potentiation on a case-by-case basis, and further states that interactions are characteristically exhibited at high concentrations and are less likely at low concentrations.

ACGIH (2015) recommends a special case method for deriving occupational exposure limits for vapors of mixtures of certain refined hydrocarbon solvents containing components that produce acute central nervous system depression and irritation of the eyes and respiratory tract. The method is based on the assumption of dose addition and the mass percent makeup of the following designated groups: C5–C6 alkanes (with the exception of n-hexane), C7–C8 alkanes, C5–C6 cycloalkanes, C7–C8 cycloalkanes, C7–C8 aromatics (with the exception of toluene), C9–C15 alkanes, C9–C15 cycloalkanes, and C9–C15 aromatics (with the exceptions of naphthalene, methylnaphthalene, and indene). More details of the method are detailed in Appendix H of ACGIH (2015) and McKee et al. (2005).

C.2. U.S. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION (OSHA)

OSHA (1993, 2001) also recommends a hazard index approach that employs the ratio of the exposure concentration to the PEL for each chemical and sums the ratios. If the sum of the ratios exceeds one, then

the exposure limit for the mixture is exceeded. OSHA does not restrict the approach to chemicals with similar effects.

C.3. U.S. NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (NIOSH)

NIOSH (1976) adopted a similar approach in recommending exposure limits for dichloromethane when carbon monoxide was also present because of the known additivity of the two chemicals with regard to formation of carboxyhemoglobin. NIOSH recommended that the sum of the ratios of each chemical to their recommended exposure limits not exceed one, and that the PELs for dichloromethane be adjusted downward when carbon monoxide levels were >9 ppm in order to keep the sum from exceeding unity. (More recent NIOSH [1992] recommendations are based on carcinogenicity.). In 2004, NIOSH introduced mixtures research agenda, showing the importance of a mixtures program to occupational exposures https://www.cdc.gov/niosh/docs/2005-106/pdfs/2005-106.pdf

C.4. U.S. CONSUMER PRODUCT SAFETY COMMISSION (CPSC)

In response to the U.S. Consumer Product Safety Improvement Act of 2008, the CPSC convened a Chronic Hazard Advisory Panel (CHAP) to study the health effects of all phthalates and phthalate alternatives as used in children's toys and child care articles (CPSC 2014). The panel report concluded that the most sensitive and most extensively studied health effect in animals from phthalates with three to seven or eight carbon atoms in the backbone of the alkyl side chain is referred to as the rat phthalate syndrome (CPSC 2014). In this syndrome, exposing pregnant rat dams causes a syndrome of anti-androgenic effects in male offspring including male reproductive tissue malformations (e.g., hypospadias), retention of nipples/areolae, and reduced anogenital distance depending on dose level and time and duration of exposure. The CPSC (2014) conducted a cumulative risk assessment for five phthalates demonstrated to cause rat phthalate syndrome effects: di(2-ethylhexyl) phthalate, dibutyl phthalate, diisobutyl phthalate, butylbenzyl phthalate, and diisononyl phthalate. The approach used a modified hazard index approach in which estimates of daily intakes of these phthalates were estimated from urine biomonitoring data for phthalate metabolites in individual pregnant women and women of reproductive age in the U.S. NHANES of 2005–2006 and individual children from 2 to 36 months of age in a study called the Study for Future Families (CPSC 2014). The estimates of daily intakes of each of the subject phthalates were divided by a "potency estimate for antiandrogenicity" for the subject phthalate (a value comparable to an RfD derived from a POD in a selected rat study divided by uncertainty factors) to derive a HQ. The hazard index for each individual subject was derived by summing the HQs for the subject phthalates. The distributions of the hazard indices for these phthalates in pregnant women and

children were assessed: about 10% of the studied pregnant women and about 5% of studied mothers and children had hazard indices >1 (CPSC 2014).

C.5. EPA OFFICE OF RESEARCH AND DEVELOPMENT (ORD)

The EPA ORD (EPA 1986) guidelines for risk assessment of chemical mixtures recommended the use of exposure and health effects data for the mixture of concern or a similar mixture if available. If not, the use of data for the components was recommended. When more than one of these approaches is feasible, EPA (1986) recommended a comparison of results from the different approaches.

The guidelines recommended the assessment of interactions data, when available, in terms of relevance to subchronic or chronic exposure and suitability for quantitatively altering the risk assessment. Interactions data were considered likely to be available mainly for pairs of chemicals, which could be assessed separately from those with no such information. The guidelines recommended, however, exploring the possibility that other components of the mixture may interfere with the interaction of the chemical pair on which quantitative interaction data are available. If interference appears likely, then quantitative alteration of the risk assessment may not be justifiable.

The assessment of the noncarcinogenic effects of the components usually proceeds by the hazard index method. Because it assumes dose additivity, the hazard index method is most suitable for chemicals with similar effects. If the mixture includes chemicals that have different effects, then EPA recommended the calculation of separate hazard indices for each end point of concern. The guidelines mentioned that if data are sufficient to derive individual acceptable levels for a spectrum of effects, the hazard index may suggest what types of effects might be expected from the mixture exposure. Subsequent guidance for Superfund risk assessment gave further explicit directions for the hazard index approach, including the combining of hazard indices for multi-route exposure and the calculation of separate hazard indices for different target organ toxicities (EPA 1989a). For carcinogenic effects, the guidelines recommended summing the risks across carcinogenic components (i.e., assume response addition).

EPA ORD developed additional mixtures guidance for risk assessment (EPA 2000), which supplemented, but did not replace, the broad principles and concepts in the original EPA ORD guidelines (EPA 1986). The supplementation emphasized an interactive and iterative problem formulation step to supplement the four parts of the EPA paradigm for assessing human health risks applied to chemical mixtures: hazard identification, dose-response assessment, exposure assessment, and risk characterization. The recommended problem formulation process involved the following three steps: (1) evaluate the nature of

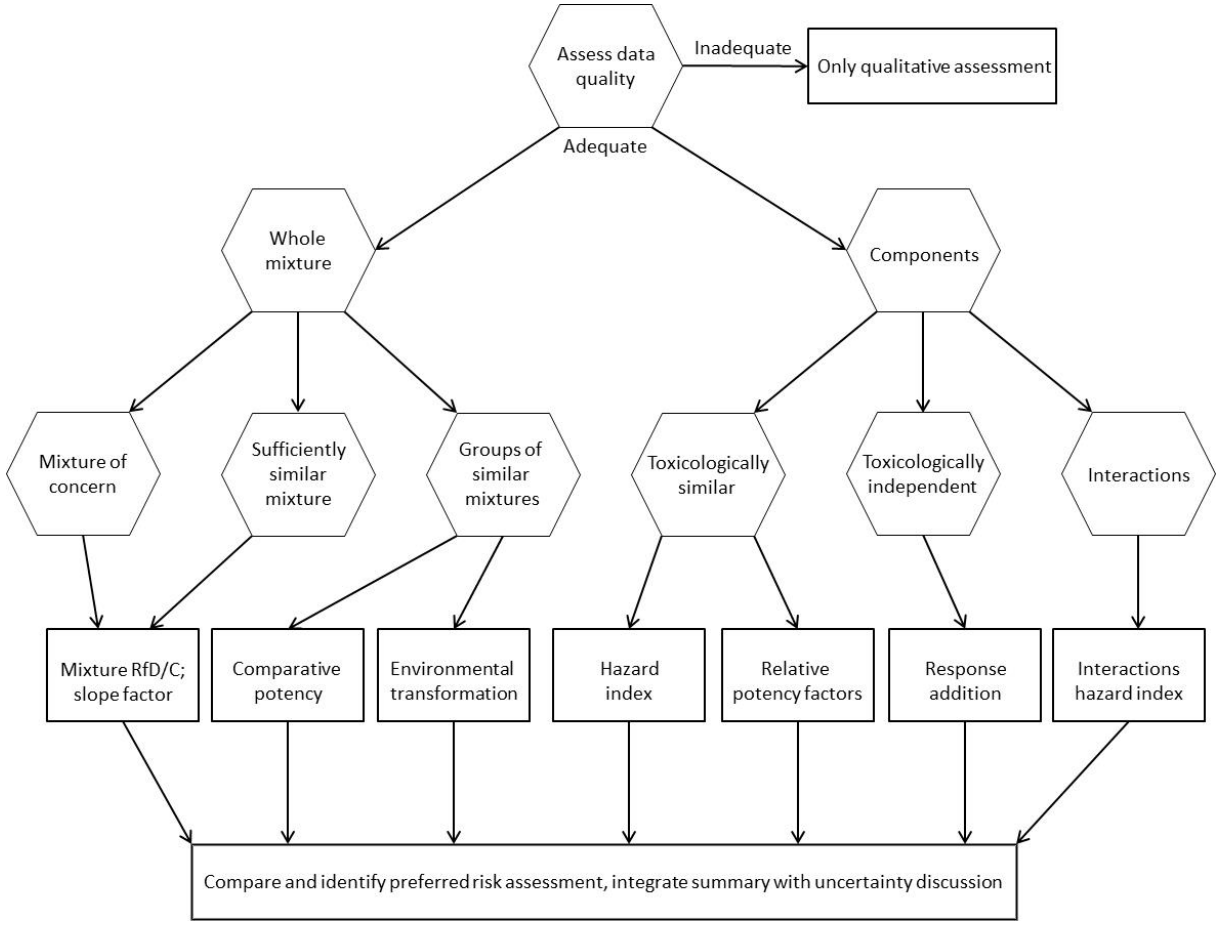
the problem; (2) define the objectives of the risk assessment; and (3) develop a data analysis and risk characterization plan. The problem formulation should: (1) assess the quality, quantity and pertinence of available information, (2) select end points to be assessed; and (3) review conceptual models that describe the relationships between exposure to the mixtures and risk for adverse health outcomes. Figure C-1 is presented to illustrate the different types of risk assessment processes that could be implemented based on the availability and quality of the data for the mixture of concern. EPA (2000) recommended that all possible assessment paths should be performed.

The guidance focused on procedures for dose-response assessment and risk characterization, noting that other EPA guidance existed to address exposure assessment and specific toxic end point evaluations. The supplemental guidance provided descriptions of methods for using whole-mixture data on a toxicologically similar mixture, methods on incorporating information on toxicologic interactions to modify a hazard index, and generalized procedures for mixtures involving classes of similar chemicals (EPA 2000). Expanded discussion was also included of concerns and uncertainties to be considered when using only whole-mixtures data (e.g., environmental transformations) or only data on the individual chemical components (e.g., the possible existence of interactions among the components—i.e., deviations from additivity).

C.6. EPA OFFICE OF AIR AND RADIATION (OAR)

The EPA OAR has completed four National Air Toxic Assessments (NATAs) for data collected in 1996, 1999, 2002, and 2005. The NATAs estimate chronic cancer risk and noncancer hazard from inhaling chemicals identified as air toxics (see EPA 2013). The assessments are based on collected ambient air concentration data for air toxics from stationary sources (e.g., large industrial facilities and smaller sources such as gasoline stations), mobile sources (e.g., cars and trucks), background sources (e.g., natural emission sources) and secondary formation (i.e., pollutants formed from other pollutants emitted in air) across broad geographic areas in the United States (e.g., counties, states). In the latest NATA, data for 177 air toxics plus diesel exhaust particulate matter were collected in 2005 and risk estimates of cancer or noncancer effects were developed for a subset of 139 chemicals with health data based on chronic exposure (cancer results for 80 air toxics and noncancer results for 110 air toxics) (EPA 2011c). Exposures were estimated from atmospheric dispersion models and human activity pattern data. Cancer risks for individual carcinogenic air toxics in outdoor air were calculated by multiplying estimates of chronic lifetime exposure levels by upper-bound inhalation unit risk estimates. Individual cancer risks

Figure C-1. EPA (2000) Description of Different Risk Assessment Paths for Chemical Mixtures Based on the Availability and Quality of the Data



EPA = Environmental Protection Agency; RfD/C = reference dose/concentration

Source: EPA 2000

were added under the assumption of response addition (independent action) to estimate cumulative cancer risks. When the risks from all carcinogenic air toxics were added, the approach estimated that there were about 3,100 census tracts (5% of about 66,000 census tracts in the United States) with increased cancer risks greater than 100 in a million. A target-organ, dose-additivity hazard index approach was used to estimate hazard potential for noncancer respiratory and neurological effects. Adequate toxicity data were available to calculate respiratory-effects HQs for 41 air toxics. The cumulative respiratory hazard indices for U.S. census tracts indicated that about 69 million people (of about 285 million U.S. residents) lived in regions with respiratory hazard indices >1.0 and about 174,000 people lived in regions with respiratory hazard indices >10. The EPA emphasized that the "NATA is a prioritization tool to identify geographic areas, pollutants and emission sources that should be evaluated further to gain a better understanding of health risks posed by air toxics."

C.7. EPA RISK ASSESSMENT FORUM

The EPA Risk Assessment Forum (EPA 2003) described a framework for conducting cumulative risk assessment, defined as "an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors." The framework does not limit "agents or stressors" to only chemicals, but includes other biological or physical agents or conditions, and specifies that "combined risk" does not mean that risks are necessarily added, but rather that some analysis should be conducted to assess how the targeted agents or stressors may interact." Three phases to the framework were described: (1) planning, scoping, and problem formulation; (2) analysis; and (3) risk characterization. The first phase entails the establishment of the goals, breadth, depth, and focus of the assessment and the production of a conceptual model that establishes the stressors to be evaluated, the health or environmental effects to be evaluated, and what is known about exposure-response relationships for the subject agents or stressors. The analysis phase includes developing exposure profiles, considering potential interactions among agents or stressors, and estimating risks to the population or populations under consideration. The end product of phase 2 is an analysis of the risks associated with the multiple agents or stressors to which the studied population or populations are exposed. The third phase evaluates the significance of the risk estimates, the reliability of the estimates and associated uncertainties, and the overall confidence in the assessment. Discussion of other details of this framework were previously discussed in Section 3.3.8 of this document.

C.8. EPA OPP

The EPA OPP is required under legislative statutes to determine with reasonable certainty that consumption of raw agricultural commodities or processed foods containing residues of a specific pesticide will not cause harm to humans, especially infants and children (EPA 2002b). The FQPA of 1996 further requires EPA to: (1) base its risk assessment for each pesticide chemical on aggregate exposure (total food, drinking water, residential, and other nonoccupational exposures); (2) consider available evidence concerning the cumulative effect on infants and children of pesticide chemicals having a common mechanism of toxicity; and (3) use an additional 10-fold margin of safety to take into account potential pre- and postnatal toxicity and completeness of the toxicity and exposure databases (EPA 2002a). This additional safety factor is often referred to as the FQPA Safety Factor.

As described earlier, the EPA OPP approach for cumulative risk assessments for pesticides sharing a common effect through a common mechanism involves: (1) determination of whether or not a group of structurally related pesticides produces a common effect by a common mechanism; (2) selection of an index chemical and determination of RPFs for members of the group; (3) determination of concentrations of member chemicals in foods and environmental media; (4) estimation of intakes for target population for multiple exposure pathways using exposure models; and (5) assessment of risks for target populations using a POD/MOE hazard indicator method when appropriate data are available (EPA 2002b).

The EPA OPP cumulative risk assessment for N-methyl carbamates provides an illustrative example of the approach. EPA (2007b) determined that N-methyl carbamate insecticides represent a common mechanism group based on similar structural characteristics and shared ability to produce neurological effects via inhibition of acetylcholine esterase (AChE) at the active enzymatic site. A multi-chemical, multi-pathway PBPK/PD model could not be developed for the cumulative risk assessment, because appropriate pharmacokinetic data for model development were only available for one carbamate insecticide, carbaryl. Based on an analysis of available data, including data collected by EPA and data submitted for registration, acute AChE inhibition, measured at the peak time of effect in rats, was determined to be the most sensitive effect from exposure to carbamates and thus, the pertinent effect of concern. A component-based RPF approach, assuming dose additivity, was used in the cumulative risk assessment. RPFs for 10 carbamates (and several carbamate metabolites—aldicarb sulfone, aldicarb sulfoxide, and 3- and 5-hydroxycarbofuran) were developed based on brain AChE inhibition data for adult rats (Table 6). The rat brain AChE inhibition data were modeled with a dose-time response model to estimate BMD_{10} values (doses at which AChE was inhibited by 10%), and the RPF values were

calculated by dividing the BMD_{10} value for the subject carbamate by the BMD_{10} value for the index carbamate, oxamyl. Oxamyl was chosen as the index chemical, because oxamyl, compared with the other nine carbamates, had the most robust data base for all three pertinent routes of exposure (oral, dermal, inhalation).

Uncertainty and extrapolation factors were incorporated into the cumulative risk assessment in two ways:

1. Adjustment of the RPF: Chemical-specific information was evaluated, when available, to determine chemical-specific inter-species uncertainty factors (animal to human extrapolation) and FQPA factors to arrive at adjusted RPF values for children and adults (Table 7). Chemical-specific FQPA factors were calculated, when appropriate data were available, by dividing an adult BMD_{10} by a pup BMD_{10} for AChE inhibition. In the absence of appropriate data, the default FQPA safety factor value of 10 was used. Chemical-specific interspecies uncertainty factors were calculated similarly when appropriate data were available to compare human BMD_{10} values for AChE inhibition with rat BMD_{10} values. In the absence of appropriate data, a default interspecies uncertainty factor of 10 was used (see Table 7).

2. Incorporation into the target MOE: A default uncertainty factor of 10 for intrahuman variability was taken as the target MOE for each of the carbamate insecticides. The PODs, used in the cumulative risk assessments to compare against exposure-based TEQs estimates were the route-specific rat $BMDL_{10}$ values for brain AChE inhibition shown in Table 8.

Table C-1. EPA RPFs for Oral, Dermal, and Inhalation Exposure to Carbamate Insecticides Based on Rat Brain Acetylcholinesterase Inhibition

Chemical	Oral RPF	Dermal RPF	Inhalation RPF
Aldicarb	4	ND[b]	ND
Aldicarb sulfone[a]	3.44	ND	ND
Aldicarb sulfoxide[a]	3.68	ND	ND
Carbaryl	0.15	0.71	0.51
Carbofuran	2.4	ND	ND
3- and 5-Hydroxycarbofuran[a]	2.4	ND	ND
Formetanate hydrochloride	2.18	ND	ND
Methiocarb	0.18	0.09	0.62
Methomyl	0.67	ND	ND
Oxamyl	1	1	1
Primicarb	0.02	ND	ND
Propoxur	0.11	0.03	0.18
Thiodicarb	0.89	ND	ND

[a]Values for aldicarb sulfone and aldicarb sulfoxide were calculated based on molecular weight conversions from aldicarb assuming equipotency to aldicarb. 3- and 5-Hydroxycarbofuran were assumed to be equipotent to carbofuran.
[b]ND = not derived due to lack of data.

EPA = Environmental Protection Agency; RPF = relative potency factor

Source: EPA 2007b

Table C-2. EPA Adjusted Oral RPFs for Children and Adults Exposed to N-Methyl Carbamates Based on Interspecies and FQPA Factors

Chemical	Oral RPF	Interspecies factor	FQPA factor for children	Adjusted RPF for children	Adjusted RPF for adults
Aldicarb	4	2	2	16	8
Aldicarb sulfone	3.44	2	2	13.8	6.9
Aldicarb sulfoxide	3.68	2	2	14.7	7.4
Carbaryl	0.15	10	1.8	2.7	1.5
Carbofuran	2.4	10	2.75	66	24
3- and 5-Hydroxycarbofuran	2.4	10	2.75	66	24
Formetanate hydrochloride	2.18	10	2.03	44	22
Methiocarb	0.18	10	10	18	1.8
Methomyl	0.67	5	3.05	10	3.3
Oxamyl	1	3	3.48	10	3
Primicarb	0.02	10	10	2	0.2
Propoxur	0.11	10	10	11	1.1
Thiodicarb	0.89	10	10	89	8.9

EPA = Environmental Protection Agency; FQPA = Food Quality and Protection Act; RPF = relative potency factor

Source: EPA 2007b

Table C-3. Oral, Dermal, and Inhalation BMD_{10} and $BMDL_{10}$ Values for Rat Brain Acetylcholinesterase Inhibition by Oxamyl, the Index Chemical for the EPA Cumulative Risk Assessment for N-Methyl Carbamates

End point	Oral	Dermal	Inhalation
BMD_{10}	0.24 mg/kg	34.91 mg/kg	0.0047 mg/L
$BMDL_{10}$	0.18 mg/kg	17.05 mg/kg	0.0038 mg/L (converted to 0.66 mg/kg)

BMD = benchmark dose; BMDL = benchmark dose limit; EPA = Environmental Protection Agency

Source: EPA 2007b

EPA (2007b) conducted route-specific cumulative risk assessments for adult and children exposures to N-methyl carbamate insecticides by incorporating the RPF values into an MOE approach applied to food, water, and residential exposure pathways. The residential pathways comprised oral, dermal, and inhalation exposures. Concentrations of carbamate residues in appropriate media (e.g., food, drinking water) were multiplied by appropriate interspecies- and FQPA-adjusted RPF values (Table 7) and summed to arrive at oxamyl-equivalent concentrations (TEQs), which were then used in exposure models to estimate oxamyl equivalent intakes (in units of mg/kg body weight) for appropriate exposure scenarios

for groups of adults and children in the general population. MOE values were calculated by dividing the appropriate oxamyl POD (e.g., the oral rat $BMDL_{10}$—Table 8—for oral exposure scenarios) by the estimated oxamyl TEQ intake. MOE values <10 were taken as values requiring some mitigation action; those >10 were assessed to be without the need for mitigation. EPA (2007b) determined total MOE values for combined estimates of food, water, and residential exposure scenarios (i.e., aggregate exposure), showing with a sensitivity analysis that the food pathway was the dominant exposure pathway for the general population.

C.9. U.S. NATIONAL ACADEMY OF SCIENCE (NAS)/NATIONAL RESEARCH COUNCIL (NRC)

In 1972, at the request of the EPA, the NAS recommended health-based stream criteria for a large number of pollutants. A component of this appraisal was multiple chemical exposure (NAS 1974). The NAS recommended a hazard index approach, whereby the sum of the ratios of the measured concentrations to the acceptable concentrations for the components was to be kept at a level equal to or lower than unity.

In 1989, at the request of EPA, The Safe Drinking Water Committee of the NRC suggested possible modifications of the then-current approaches for estimating the toxicity of mixtures in drinking water (NRC 1989). The NRC suggested that mixture components be grouped by end point, such as specific organ toxicity and carcinogenicity in order to assess their combined risk or hazard.

For noncancer end points, the NRC (1989) suggested a modified hazard index that sums similar toxicities and an uncertainty factor for possible synergism, depending on the information regarding interactions and the concentrations of the components. The uncertainty factor could range from one to 100. If information regarding potential interactions is available and suggests that interactions are not likely, or if the concentrations are low, the uncertainty factor could be set at one. The NRC also suggested that separate hazard indices be calculated for each toxic end point, including those that occur at higher exposure levels than the end point that is the basis for the acceptable exposure level for a component. A weighting factor would be applied to account for the lesser sensitivity of the other end points, unless an acceptable exposure level for the other end points was available. The method is similar to the TTD modification of the hazard index method, discussed previously, except that the NRC further suggested summing the hazard indices across all toxic end points.

For carcinogenic end points, the NRC (1989) concluded that it was appropriate to sum the risks (response addition with completely negative correlation of tolerances) for low-dose exposure to a mixture of carcinogens (doses with relative risks of <1.01).

The NRC (2004a) report, *Air Quality Management in the United States*, recommended that the EPA address multiple pollutants in its National Ambient Air Quality Standards (NAAQS) review and standard setting process. However, the committee making this recommendation did not "believe that the science has evolved to a sufficient extent to permit the development of multipollutant NAAQS, it would be scientifically prudent to begin to review and develop NAAQS for related pollutants in parallel and simultaneously." In response to this recommendation, EPA convened a public workshop in 2011 to discuss scientific issues and data gaps related to adopting multipollutant science and risk assessment approaches for priority hazardous air pollutants identified by the EPA under the Clean Air Act (Johns et al. 2012). The major conclusion from the workshop called for the development and adoption of a framework and methods for conducting multipollutant science and risk assessments of the well-studied priority air pollutants (Johns et al. 2012).

The NRC (2008) report, *Phthalates and Cumulative Risk Assessment: Tasks Ahead*, recommended that the EPA should conduct a cumulative risk assessment for phthalates using the dose-addition concept to all phthalates that have anti-androgenic activity. This recommendation was based on studies examining effects on developing male reproductive end points in rats after oral exposures to mixtures of phthalates or phthalates plus other anti-androgenic compounds showing that dose-addition models provided adequate fit to observed dose-response data (Christiansen et al. 2009; Hass et al. 2007; Howdeshell et al. 2007, 2008; Rider et al. 2008; see Section 3.3.1.2 *Evidence to Support or Refute the Use of Default Dose-Additivity Approaches*). To date, EPA has not conducted a cumulative risk assessment for phthalates or other anti-androgenic chemicals, but CPSC (2014) published a cumulative risk assessment for anti-androgenic effects from five phthalates, using a modified hazard index approach (see Section C.4).

C.10. U.S. DEPARTMENT OF ENERGY (DOE)

To estimate potential health effects to workers and the public exposed to unplanned release of mixtures of chemicals, the U.S. DOE followed the principles of the EPA (1986, 2000) chemical mixtures guidelines to establish a chemical mixtures methodology using a dose-additive hazard index approach (Yu et al. 2010, 2013). In this method, a hazard index (comparable to EPA's HQ) for each chemical in the mixture is calculated that is the ratio of the exposure concentration for the component "at a given receptor site" to

the toxicity guidance value for the component. Exposure concentrations are estimated with Gaussian atmospheric dispersion models that calculate exposure concentrations based on the amount of each chemical in the mixture available for release to the atmosphere, the manner of release (e.g., spill or explosion), the chemical and physical properties of each chemical, the time within the release plume, release event parameters, and meteorological conditions. The default, screening-level approach uses Protective Action Criteria (PACs) that are determined from (in order of priority) Acute Exposure Guideline Level (AEGL) values, Emergency Response Planning Guideline (ERPG) values or Temporary Emergency Exposure Limit (TEEL) values. Different PAC benchmark values are established to indicate threshold concentrations for increasing severity of acute effects (i.e., levels below the threshold value are not expected to produce effects of the indicated severity): PAC-0, threshold for no adverse effects; PAC-1 threshold for mild or transient effects; PAC-2, threshold for irreversible or serious effect that could impair a person's ability to take protective actions; and PAC-3: threshold for life-threatening effects. As an initial screen to provide protection for first responders using this method, a cumulative hazard index is calculated by summing individual component hazard indices using PAC-1 or PAC-2 values for all known components in the mixtures, regardless of their target organ or expected MOA; cumulative hazard indices >1 indicate risks from acute exposure to the mixture and the need to take some mitigating action. A refinement to the screening level cumulative hazard index approach is analogous to the TTD method described in Section 3.3.3 of this document, in which cumulative hazard indices for chemicals in the mixture producing the same or similar effects are calculated. All chemicals in the DOE database with PACs (approximately 3,300 chemicals) are assigned any number of 60 health code numbers as guided by available toxicity data for the individual chemical (see Table 1 in Yu et al. 2013). Some of the health code numbers are for acute effects, others are for chronic effects. The health code numbers are used to group individual hazard indices for chemicals in the mixture of concern affecting the same or similar target organs, which are then used to calculate cumulative hazard indices for specific target organs or effects. The approach assumes that acute and chronic effects are independent and for a specific target organ calculates separate cumulative hazard indices for acute and chronic effects. For example, separate cumulative hazard indices are calculated for acute reproductive effects (health code number, 5.00) and chronic reproductive effects (health code number, 5.11). Yu et al. (2013) reported that activities were ongoing to evaluate modifications to the target organ/effect hazard index approach. The modifications under consideration involved the development of weighting factors for target organ hazard indices based on rankings of health code numbers for each type of PAC value.

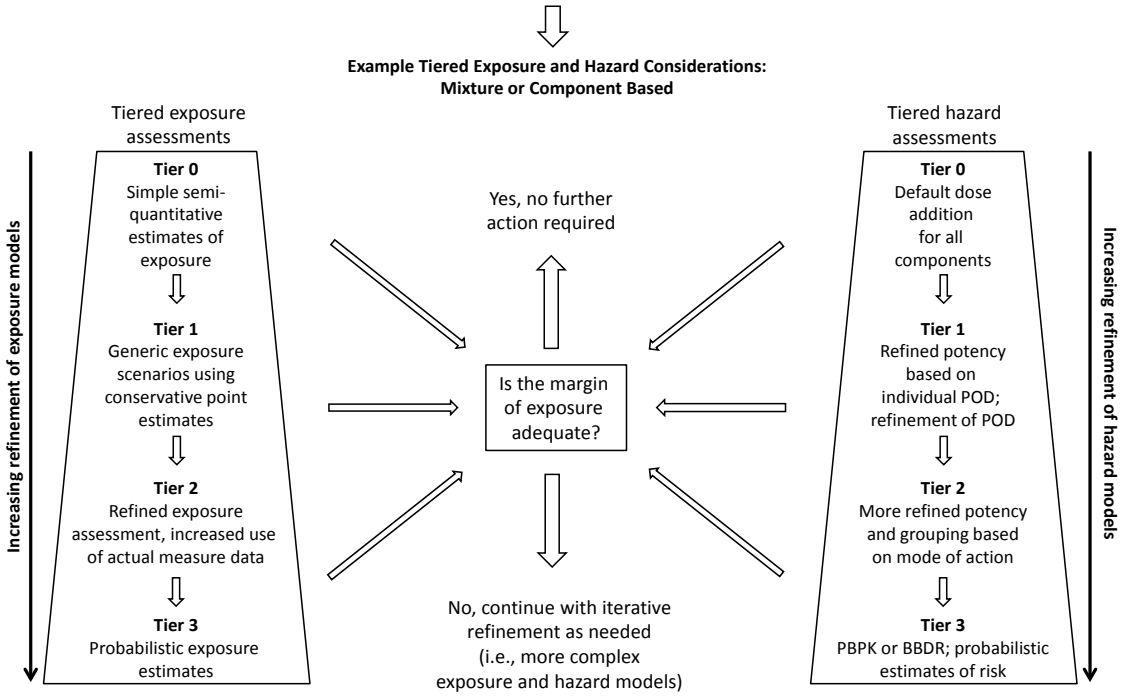
C.11. WORLD HEALTH ORGANIZATION (WHO)/INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY (IPCS)

Based on a workshop convened by the WHO/IPCS, a framework for assessing health risks from combined exposure to multiple chemicals has been described (Meek 2013; Meek et al. 2011). The framework entails tiered parallel assessments of exposure and hazard that increase in refinement and data depth and are accompanied by an iterative problem formulation process that asks key questions about the nature and likelihood of exposure to multiple chemicals and the rationale for considering multiple chemicals in an assessment group (Figure C-2). Within each tier of assessment, information on exposure and hazard from the mixture are brought together to assess the MOE (the ratio of some POD assessment (NOAEL/LOAEL or BMD_{10}) of combined toxicity to an estimate of exposure). From this MOE assessment, decisions are made about continuing (or not) with iterative refinements based on the availability of appropriate data and scope of the assessment. Tier 0 of the framework calls for simple semi-quantitative estimates of exposure and a default dose-addition hazard approach for all components of the mixture. Tier 1 calls for refined exposure scenarios using conservative point estimates and refined PODs. Tier 2 calls for increased use of actual data in the exposure assessment and more refined potency and groupings based on MOA. Tier 3 calls for probabilistic exposure estimates and refined probabilistic estimates of hazard risk involving PBPK and PBPK/PD modeling when appropriate. The tiered approach allows consideration of the efficiency of use of resources: each progressive tier is more refined, requiring more labor and data (Meek 2013).

A case study applied the WHO/IPCS framework to conduct a screening-level assessment under the Canadian Environmental Protection Act of a group of seven polybrominated diphenyl ethers (PBDEs) present in commercial mixtures used as flame retardants in a number of consumer products (Meek et al. 2011). In the Tier 0 assessment, semi-quantitative measures of exposure was determined through comparison of relative rankings, physicochemical properties, and use patterns with those for congeners with deterministic estimates of exposure. In the absence of toxicity guidance values for the individual congeners, a hazard index could not be calculated, but the summation of semiquantitative estimates of exposure was greater than the LOAEL for the most toxic congener with toxicity data. This simplified MOE was used to prompt a Tier 1 assessment. In the Tier 1 assessment, upper-bounding estimates of total intakes of PBDEs were developed based on maximum levels in air, water, dust, foods, and human breast milk and reference intake values for six age groups within the Canadian population. Based on review of available results from animal toxicity tests with several of the individual congeners or commercial PBDE mixtures identifying effects on liver, thyroid, and neurological (behavioral) development in neonatal mice, a LOAEL of 0.8 mg/kg body weight/day for effects on locomotion,

rearing, and total activity was selected as the critical (most sensitive) POD. A MOE of about 300 was calculated based on a comparison of this POD with the upper bounding intake estimate of 0.0026 mg/kg body weight/day. Descriptions and considerations of the uncertainties in the Tier 1 exposure and dose-response assessments were used to make recommendations for additional risk assessment and research activities. Assessments at the Tier 2 or 3 levels were not conducted (Meek et al. 2011).

Figure C-2. World Health Organization/International Programme on Chemical Safety Framework for Evaluating the Risk of Combined Exposure to Multiple Chemicals



BBDR = biologically based dose-response; PBPK = physiologically based pharmacokinetic; POD = point of departure

Source: Meek et al. 2011

C.12. HEALTH COUNCIL OF NETHERLANDS

The Health Council of Netherlands published a report outlining a decision flow chart to guide hazard identification and risk assessments for chemical mixtures (Figure C-3). The approach was developed to guide hazard identification and risk assessment for specified combinations of chemicals, as well as for more complex mixtures that may have components that are unknown. The flow diagram recommended using toxicity data on the mixture of concern or a "comparable mixture" if data are available, and using toxicity data on constituents (i.e., components) if toxicity data for the mixture itself or a comparable mixture are not available. For the component-based approaches, the decision tree called for determining whether or not few components or many components were present in the mixture.

For the case of many components, a step was recommended to decide whether or not a few components of most concern (based on a combination of severity of hazard and concentration in the mixture) should be used to estimate toxicity of the mixture, or a grouping or lumping approach such as that described by Verhaar et al. (1997) should be used. Continuing in the component-based path, the approach called for grouping chemicals with similar toxicological action, assessing information on possible interactions and using dose addition approaches for assessing risks. For components with dissimilar toxicological action, a response-addition approach was recommended, using exposure limits for individual agents for risk assessment. To evaluate evidence for interactions among components of the mixture, the approaches described by Mumtaz and Durkin (1992) and EPA (2000) were recommended.

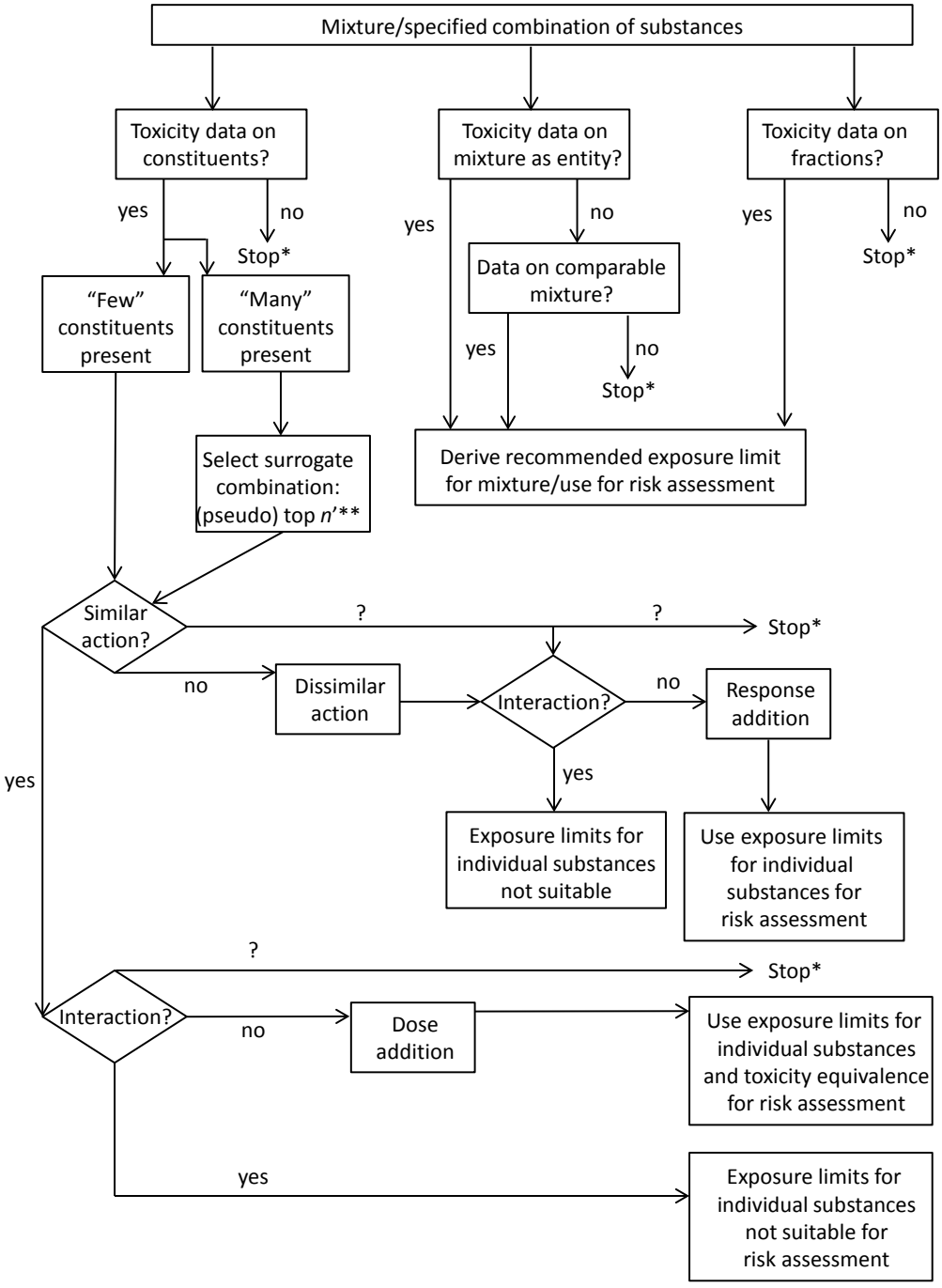
C.13. NORWEGIAN SCIENTIFIC COMMITTEE FOR FOOD SAFETY

The Norwegian Scientific Committee for Food Safety (2013) published a report, *Combined Toxic Effects of Multiple Chemical Exposures*, outlining a decision-tree flow chart for use in human health risk assessments of chemical mixtures or concurrent exposure. The flow chart did not suggest approaches for the cases where toxicity data may exist for the mixture of concern or a similar mixture. Two key opinions incorporated into the flow chart, which represents a component-based approach (see Figure C-4), are as follows:

1. For chemicals with similar MOAs, adverse effects from multiple exposures occur due to dose addition, even if exposures to components are below their respective acceptable or tolerable daily intakes.

2. For chemicals with dissimilar MOAs, adverse effects from multiple exposures are not expected when the exposures to the individual components are below the respective acceptable or tolerable daily intakes. When compounds in the mixture are thought to act independently of each other, the recommended approach is a chemical-specific approach for each component, if pertinent toxicity data are available.

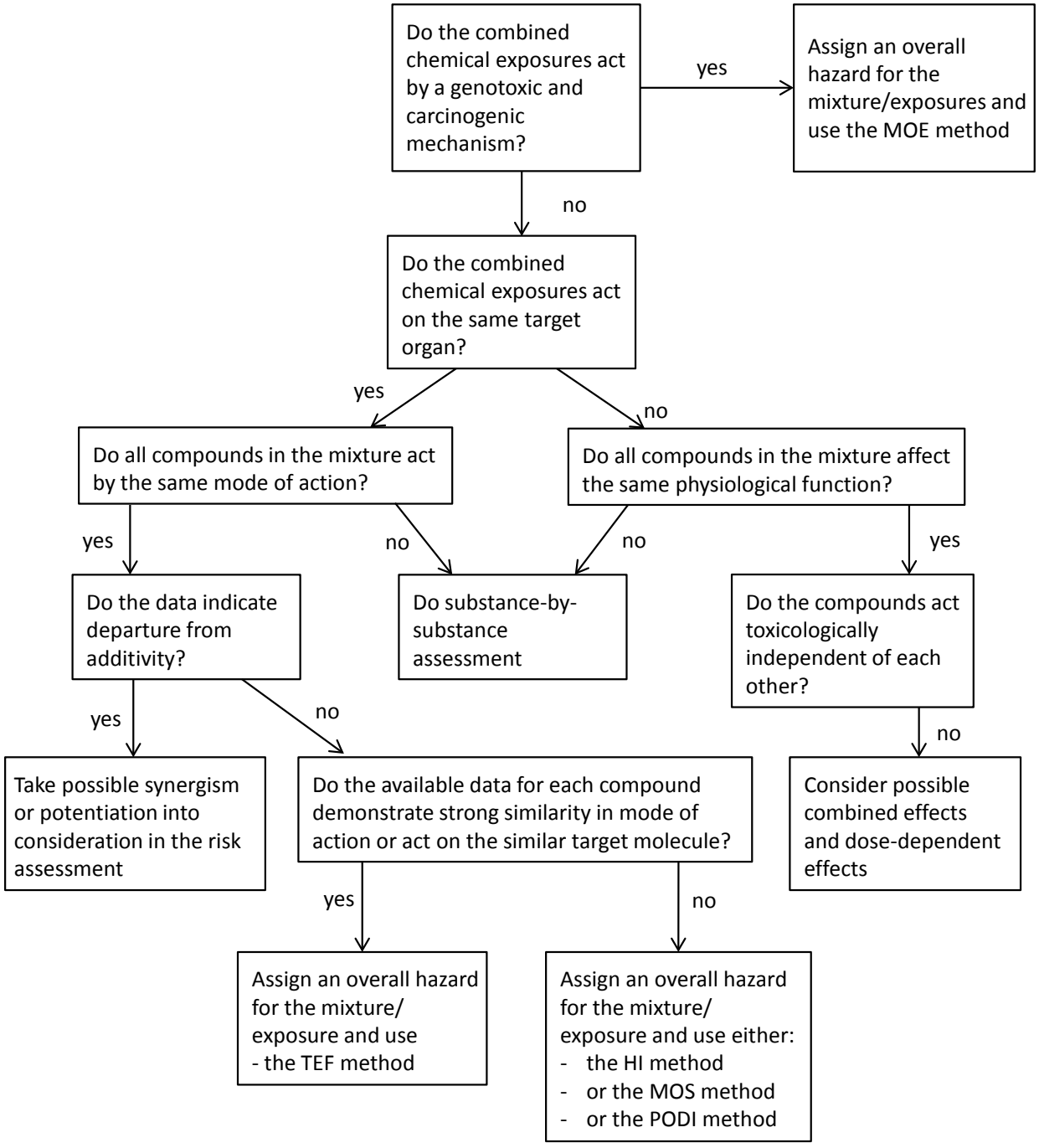
Figure C-3. Health Council of Netherlands Framework for Evaluating the Risk of Combined Exposure to Multiple Chemicals



*Stop = data required to complete the evaluation.
**"Top *n*" and "pseudo top *n*;" *n* represents the most "risky" chemicals or groups of chemicals, respectively.

Source: Feron et al. 2004 (Reprinted from Environmental Toxicology and Pharmacology, 18(3):215-222. Copyright (2004), with permission from Elsevier)

Figure C-4. Norwegian Scientific Committee for Food Safety Flow Chart for Use in Risk Assessments of Multiple Chemical Exposures



HI = hazard index; MOE = margin of exposure; MOS = margin of safety; PODI = point of departure index; TEF = toxic equivalency factor

Source: Norwegian Scientific Committee for Food Safety 2013

C.14. DANISH MINISTRY OF THE ENVIRONMENT

The Danish Ministry of the Environment (DEPA 2009) convened a workshop to examine existing scientific knowledge on combination effects of endocrine disrupters, with a focus on regulatory aspects. Several consensus recommendations were made by workshop participants:

1. "Cumulative risk assessment for endocrine disrupters was seen as both necessary and feasible. The predominant chemical-by-chemical approach in risk assessment was regarded as insufficiently protective against the possibility of mixture effects/ effects of combined exposure.

2. The application of dose (or concentration) addition as an assessment method was recommended as a default, until evidence as to the suitability of alternative assessment concepts emerges.

3. A pre-occupation with mechanisms or modes of action as the starting point for the grouping of endocrine disrupters into classes to be subjected to mixtures risk assessment was seen as not practical and scientifically hard to justify. Instead, grouping criteria should focus on common health related effects and the likelihood of co-exposures.

4. The full potential of cumulative risk assessment for endocrine disrupters cannot be reached without filling a number of data gaps, most importantly in the area of mixtures exposure assessment.

5. An enhancement of the legal framework in Europe with a view to mandating cumulative risk assessment should be given serious consideration."

C.15. EUROPEAN FOOD SAFETY AUTHORITY

The European Food Safety Authority's Panel on Plant Protection Products and their Residues evaluated existing methodologies for assessing risks of exposure to two or more pesticides in combination and made recommendations for refining the methodologies (EFSA 2008). The panel noted that their recommendations were for component-based approaches for groups of pesticides producing common adverse outcomes or MOAs. For mixtures of pesticides with different targets or MOAs, the panel concluded that there was no evidence for dose additivity at low exposure levels below toxicological reference values, and that risk associated with such mixtures are determined by the component with the highest HQ. This conclusion is consistent with the concept of response addition (i.e., independent action) for these cases. The panel also concluded that no more than dose additive effects were expected for pesticides with a common target or MOA, noting that: (1) pesticide residues are generally below individual NOAELs and (2) "although toxic interactions from pesticide residues cannot be ruled out, there

is no empirical evidence for their occurrence at the expected levels of exposure from pesticide residues in food." The recommendations call for:

1. A tiered parallel assessment of exposure and hazard, conducting iterative, increasingly complex and data-intensive risk assessments by MOE comparisons between exposure and hazard estimates using increasingly data intensive methods (i.e., methods of increasing refinement).

2. Initially identifying the common assessment group "in broad terms," evaluating the evidence for common adverse outcomes or MOAs, and refining the group with more refined assessments of hazard.

3. Dose-additive, hazard index approaches, initially for all components or components with common effects and proceeding through more refined approaches involving RPFs derived from NOAELs or BMDs and, finally, RPFs refined by PBPK/PD models, if appropriate models are available.

4. Tiered exposure assessments, initially using deterministic modeling approaches, proceeding through probabilistic modeling approaches if appropriate data are available.

5. Recognition and articulation of uncertainties in both the exposure and hazard assessment portions of the risk assessment, noting a qualitative scheme for evaluating sources of uncertainties that may cause small, medium, or large, over- or under-estimation of risk. The panel noted that sources with large uncertainty potential warrant sensitivity analysis and "provide the greatest scope for refinement of the assessment."

In a related Scientific Opinion, the Panel on Plant Protection Products and their Residues evaluated existing methods for assessing chemicals acting by dissimilar MOAs and recommended dose-addition approaches for the assessment of multiple pesticides with dissimilar MOAs, provided that they produce a common adverse outcome (EFSA 2013). The recommendation to group pesticides with common adverse outcomes together in common assessment groups and use dose addition to assess cumulative risk was viewed as a pragmatic and conservative default approach. The use of a default dose addition model, regardless of mechanism of action, was also recommended by an EFSA colloquium convened to harmonize of human and ecological risk assessment of combined exposure to multiple chemicals (EFSA 2015). This default approach was considered conservative and health-protective during "lower tier" assessments, and that the default approach could be modified to include more-than- or less-than-additive predictions if adequate data were available in "higher tier" analyses. However, the colloquium concluded that further development of available tools (e.g., PBPK models, quantitative structure-activity relationship models, adverse outcome pathways, etc.) are needed prior to routine integration of these models in human and ecological risk assessment.

C.16. EUROPEAN COMMISSION NON-FOOD SCIENTIFIC COMMITTEES

The European Commission non-food scientific committees (Scientific Committee on Consumer Safety, Scientific Committee on Health and Environmental Risks, Scientific Committee on Emerging and Newly Identified Health Risks) reviewed available scientific literature on the toxicity of chemical mixtures, drew conclusions from the review, and proposed a decision tree flow chart for evaluating the risk of chemical mixtures (EC 2012). Conclusions reached included the following:

1. "Chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly. These effects can be described by dose/concentration addition.

2. For chemicals with different modes of action (independently acting), no robust evidence is available that exposure to a mixture of such substances is of health or environmental concern if the individual chemicals are present at or below their zero-effect levels.

3. Interactions (including antagonism, potentiation, and synergies) usually occur at medium or high dose levels (relative to the lowest effect levels). At low exposure levels, they are either unlikely to occur or are toxicologically insignificant.

4. In view of the almost infinite number of possible combinations of chemicals to which humans and environmental species are exposed, some form of initial filter to allow a focus on mixtures of potential concern is necessary. Several criteria for such screening are offered.

5. With regard to the assessment of chemical mixtures, a major knowledge gap at the present time is the lack of exposure information and the rather limited number of chemicals for which there is sufficient information on their mode of action. Currently, there is neither an agreed inventory of mode of actions, nor a defined set of criteria [on] how to characterize or predict mode of action for data-poor chemicals.

6. If no mode of action information is available, the dose/concentration addition method should be preferred over the independent action approach. Prediction of possible interaction requires expert judgement and hence needs to be considered on a case-by-case basis."

The decision tree flow chart for evaluating chemical mixtures, illustrated in Figure C-5, calls for:

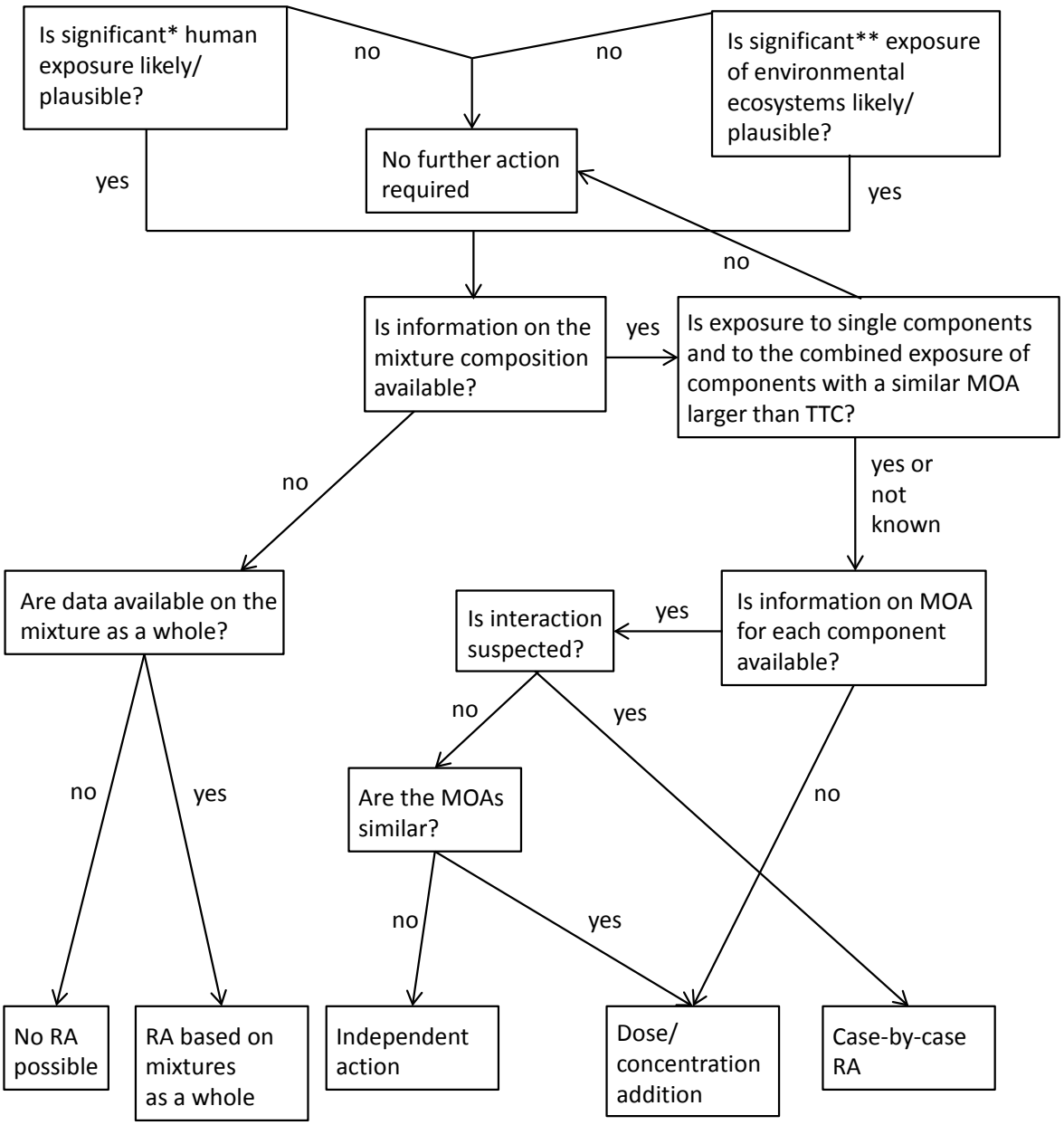
1. An initial assessment that significant human exposure is likely or plausible. Significance of exposure was to be determined by the frequency, duration, and magnitude of exposure.

2. Utilization of toxicity data on the mixture as a whole if available.

3. Use of dose-addition approaches if the mixture components produce common effects via a common MOA, and response addition approaches if mixture components are known to act independently.

4. Use of dose-addition approaches as a default approach if MOA information is not available.

Figure C-5. European Commission Non-food Scientific Committees' Recommended Decision Tree for Assessing Risks from Chemical Mixtures



*"Significant" exposure is determined by the frequency, duration, and magnitude of exposure.
**For the environment, an exposure-driven assessment without at least a preliminary risk characterization, as well as the TTC model, is hardly acceptable. Therefore, it must be considered as significant any exposure produced by emissions capable to modify the natural background conditions.
***Evidence for interaction can be found at various steps of the decision tree (e.g., comparing product information with compound-based assessment).

MOA = mode of action; RA = risk assessment; TTC = threshold of toxicological concern

Source: EC 2012 (© European Union, 1995–2015)

C.17. REFERENCES

ACGIH. 1984. Threshold limit values-discussion and thirty-five year index with recommendations. Annals of the American Conference of Governmental Industrial Hygienists. American Conference of Governmental Industrial Hygienists, 365-368.

ACGIH. 2015. Appendix E: Threshold limit values for mixtures, Appendix H: Reciprocal calculation method for certain refined hydrocarbon solvent vapor mixtures. In: TLVs and BEIs based on the documentation of the threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 80-82, 92-96.

Christiansen S, Scholze M, Dalgaard M, et al. 2009. Synergistic disruption of external male sex organ development by a mixture of four antiandrogens. Environ Health Perspect 117(12):1839-1846. 10.1289/ehp.0900689.

CPSC. 2014. Chronic hazard advisory panel on phthalates and phthalate alternatives (with appendices). Bethesda, MD: U.S. Consumer Product Safety Commission. http://www.cpsc.gov/PageFiles/169902/CHAP-REPORT-With-Appendices.pdf. May 8, 2015.

DEPA. 2009. Expert workshop on combination effects of chemicals, 28-30 January 2009, Hornbaek, Denmark. Danish Ministry of the Environment, Danish Environmental Protection Agency. http://www.food.dtu.dk/~/media/Institutter/Foedevareinstituttet/Publikationer/Pub-2009/2009%20bilag_2_expertworkshop.ashx?la=da. May 14, 2015.

EC. 2012. Toxicity and assessment of chemical mixtures. European Commission. Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), and Scientific Committee on Consumer Safety (SCCS). http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf. July 10, 2015.

EFSA. 2008. Opinion of the Scientific Panel on Plant Protection products and their residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005. European Food Safety Authority. EFSA J 704:1-84. http://www.efsa.europa.eu/en/efsajournal/doc/705.pdf. May 8, 2015.

EFSA. 2013. International frameworks dealing with human risk assessment of combined exposure to multiple chemicals. European Food Safety Authority. EFSA J 11(7):3313. http://www.efsa.europa.eu/de/efsajournal/doc/3313.pdf. July 29, 2015.

EFSA. 2015. Summary report EFSA Scientific Colloquium 21. Harmonisation of human and ecological risk assessment of combined exposure to multiple chemicals 11-12 September 2014. European Food Safety Authority. EFSA Supporting Publications 12(3). http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2015.EN-784/pdf. November 8, 2016.

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

EPA. 1989a. Risk assessment guidance for superfund. Volume I. Human health evaluation manual (Part A). Washington, DC: U.S. Environmental Protection Agency, Office of Emergency and Remedial Response. EPA5401859002. http://www.epa.gov/oswer/riskassessment/ragsa/pdf/rags_a.pdf. July 22, 2015.

EPA. 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. Washington, DC: U.S. Environmental Protection Agency. EPA630R00002. http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=4486. July 2, 2015.

EPA. 2002a. Consideration of the FQPA safety factor and other uncertainty factors in cumulative risk assessment of chemicals sharing a common mechanism of toxicity. Washington, DC: U.S. Environmental Protection Agency. http://www.epa.gov/oppfead1/trac/science/APPS-10X-SF-for-CRA.pdf. May 8, 2015.

EPA. 2002b. Guidance on cumulative risk assessment of pesticide chemicals that have a common mechanism of toxicity. U.S. Environmental Protection Agency. http://www.epa.gov/oppfead1/trac/science/cumulative_guidance.pdf. May 8, 2015.

EPA. 2003. Framework for cumulative risk assessment Washington, DC: U.S. Environmental Protection Agency. EPA600P02001F. http://www2.epa.gov/sites/production/files/2014-11/documents/frmwrk_cum_risk_assmnt.pdf. May 8, 2015.

EPA. 2007b. Revised n-methyl carbamate cumulative risk assessment. U.S. Environmental Protection Agency. http://www.epa.gov/oppsrrd1/reregistration/REDs/nmc_revised_cra.pdf. May 14, 2015.

EPA. 2011b. Summary of results for the 2005 national scale assessment. Technology transfer network air toxics 2005 National-scale air toxics assessment. U.S. Environmental Protection Agency. http://www.epa.gov/ttn/atw/nata2005/05pdf/sum_results.pdf. July 20, 2015.

EPA. 2013. National air toxics assessments. Technology transfer network air toxics web site. U.S. Environmental Protection Agency. http://www.epa.gov/airtoxics/natamain. July 20, 2015.

Feron VJ, van Vliet PW, Notten WR. 2004. Exposure to combinations of substances: A system for assessing health risks. Environ Toxicol Pharmacol 18(3):215-222. 10.1016/j.etap.2003.11.009.

Hass U, Scholze M, Christiansen S, et al. 2007. Combined exposure to anti-androgens exacerbates disruption of sexual differentiation in the rat. Environ Health Perspect 115(Suppl 1):122-128. 10.1289/ehp.9360.

Howdeshell KL, Furr J, Lambright CR, et al. 2007. Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: Altered fetal steroid hormones and genes. Toxicol Sci 99(1):190-202. 10.1093/toxsci/kfm069.

Howdeshell KL, Wilson VS, Furr J, et al. 2008. A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicol Sci 105(1):153-165. 10.1093/toxsci/kfn077.

Johns DO, Stanek LW, Walker K, et al. 2012. Practical advancement of multipollutant scientific and risk assessment approaches for ambient air pollution. Environ Health Perspect 120(9):1238-1242. 10.1289/ehp.1204939.

McKee RH, Medeiros AM, Daughtrey WC. 2005. A proposed methodology for setting occupational exposure limits for hydrocarbon solvents. J Occup Environ Hyg 2(10):524-542. 10.1080/15459620500299754.

Meek ME. 2013. International experience in addressing combined exposures: Increasing the efficiency of assessment. Toxicology 313(2-3):185-189. 10.1016/j.tox.2012.09.015.

Meek ME, Boobis AR, Crofton KM, et al. 2011. Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regul Toxicol Pharmacol 60:S1-S14. 10.1016/j.yrtph.2011.03.010.

Mumtaz MM, Durkin PR. 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicol Ind Health 8(6):377-406.

NAS. 1974. Water quality criteria, 1972. Section III- Freshwater aquatic life and wildlife: Mixtures of two or more toxicants. National Academy of Sciences, National Academy of Engineering. II-XIX, 1-4, 106-108, 122-123. EPAR373033.

NIOSH. 1976. Criteria for a recommended standard for occupational exposure to methylene chloride. Cincinnati, OH: National Institute for Occupational Safety and Health.

NIOSH. 1992. NIOSH recommendation for occupational safety and health. Compendium of policy documents and statements. Cincinnati, OH: National Institute of Occupational Safety and Health.

Norwegian Scientific Committee for Food Safety. 2013. Combined toxic effects of multiple chemical exposures. Oslo: Vitenskapskomiteen for mattrygghet. Norwegian Scientific Committee for Food Safety. Doc. No.: 11/005-final. http://www.vkm.no/dav/906de6c1a6.pdf. July 21, 2015.

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

NRC. 2004a. Executive summary. In: Air quality management in the United States. National Research Council, Committee on Air Quality Management in the United States. http://www.nap.edu/catalog/10728.html. June 30, 3015.

NRC. 2008. Summary. In: Phthalates and cumulative risk assessment: The tasks ahead. Committee on the Health Risks of Phthalates, National Research Council. http://www.ncbi.nlm.nih.gov/pubmed/25009926. July 22, 2015.

OSHA. 1993. Air contaminants. 29 CFR Part 1910. U.S. Department of Labor. Occupational Safety and Health Administration. Fed Regist 58(124):35338-35351.

OSHA. 2001. Air contaminants. Subpart Z. Toxic and hazardous substances. Occupational Safety and Health Administration. Code of Federal Regulations 29 CFR 1910.1000. http://www.gpo.gov/fdsys/pkg/CFR-2001-title29-vol6/pdf/CFR-2001-title29-vol6-sec1910-1000.pdf. July 14, 2015.

Rider CV, Furr J, Wilson VS, et al. 2008. A mixture of seven antiandrogens induces reproductive malformations in rats. Int J Androl 31(2):249-262. 10.1111/j.1365-2605.2007.00859.x.

Verhaar HJ, Morroni JR, Reardon KF, et al. 1997. A proposed approach to study the toxicology of complex mixtures of petroleum products: the integrated use of QSAR, lumping analysis and PBPK/PD modeling. Environ Health Perspect 105(Suppl 1):179-195.

Yu XY, Glantz CS, Yao J, et al. 2013. Enhancing the chemical mixture methodology in emergency preparedness and consequence assessment analysis. Toxicology 313(2-3):174-184. 10.1016/j.tox.2012.10.011.

Yu XY, Petrocchi AJ, Craig DK, et al. 2010. The development and application of the chemical mixture methodology in analysis of potential health impacts from airborne release in emergencies. J Appl Toxicol 30(6):513-524. 10.1002/jat.1558.