

3. OPTIONS AND ISSUES FOR ASSESSING HEALTH IMPACTS OF EXPOSURES TO MULTIPLE CHEMICALS AND OTHER STRESSORS

In general, assessments of health impacts or risks associated with exposures to multiple chemicals and other stressors use one of three approaches: (1) use exposure data and epidemiologic and toxicologic data for the actual mixture of concern; (2) use data for a sufficiently similar mixture; or (3) use data on the components of the mixture. Background information on these approaches is discussed in this chapter in order of preference.

3.1. MIXTURE OF CONCERN APPROACH

When exposure data and health effects data are available for the mixture of concern, use of these data has traditionally been the preferred approach (EPA 1986, 2000, 2003; see Chapter 2 for ATSDR approach and Chapter 4 for recommendations from other agencies). However, data on the mixture of concern are rarely available. When available, such data tend to be for complex mixtures that are considered a health hazard because they are generated in large quantities and are thought to cause adverse health effects. In addition, the exposures of concern generally occur at the source of the mixture.

Examples of complex mixtures with sufficient data for hazard identification and, in some cases, dose-response assessment include coke oven emissions, diesel engine exhaust, and manufactured gas emissions and residues.

- Coke oven emissions were determined by EPA to be carcinogenic to humans based on increased risk of mortality from cancer of the lung, trachea, bronchus, and other tissue sites in workers exposed to coke oven emissions as well as increased tumors in animals exposed by inhalation to aerosols of condensates of coke oven emissions (EPA 1984). Based on an analysis of respiratory cancer mortality data and exposure data for a cohort of steel workers, EPA estimated that lifetime exposure to a concentration of 0.2 $\mu\text{g}/\text{m}^3$ benzene-soluble organic material from coke oven emissions would produce a 1/100,000 extra risk of dying from respiratory cancer (EPA 1984). Based on recent epidemiological and mechanistic studies, the International Agency for Research on Cancer (IARC) determined in 2012 that sufficient evidence was available to determine that coke production is carcinogenic to humans. IARC's (2012a) determination was based on sufficient evidence for a causal relationship with lung cancer in occupationally exposed workers. IARC (2012a) also determined that there was: (1) sufficient evidence in experimental animals for the carcinogenicity of samples of tar taken from coke ovens; (2) strong evidence for a genotoxic

mechanism involving mutagenic PAHs based on both human and experimental animal studies; and (3) suggestive evidence that multiple mechanisms, including epigenetic mechanisms, may be involved in the carcinogenic response to coke oven emissions.

- Diesel engine exhaust was determined by EPA (2002c) to be likely carcinogenic to humans by inhalation based on: (1) strong, but less than sufficient, evidence for a causal association between diesel exhaust exposure and increased lung cancer risk in workers from varied occupations with diesel exhaust exposure; (2) supporting positive results in genotoxicity tests with diesel exhaust and organic constituents; (3) knowledge that a number of components of diesel exhaust have produced positive results in genotoxicity and carcinogenicity tests; and (4) positive results in cancer bioassays with rodents exposed to high intratracheal instillation doses of whole diesel exhaust, in skin painting studies using extracts of organic whole diesel exhaust, and in many chronic inhalation rat studies showing a positive lung cancer response at high exposures. A quantitative estimate of cancer risk, however, was not developed, because of inadequate exposure-response data from human studies and a determination that doses at which toxicity was observed in rats were much higher than expected environmental exposure levels” (EPA 2002c). A chronic inhalation RfC of 5 $\mu\text{g}/\text{m}^3$ was derived based on a rat NOAEL of 0.46 mg/m^3 for pulmonary inflammation transformed to a human equivalent concentration using a deposition and clearance model for diesel particulate matter and divided by uncertainty factors of 3 to account for residual interspecies extrapolation uncertainties and 10 for human variability (EPA 2002c). After review of recent epidemiology data, IARC (2014) determined that diesel engine exhaust is carcinogenic to humans based on sufficient evidence in humans indicating a causal relationship with lung cancer and a positive association with urinary bladder cancer. IARC (2014) also determined that there was sufficient evidence for carcinogenicity of whole diesel engine exhaust and particulate matter in experimental animals, but inadequate evidence for carcinogenicity of gas-phase diesel engine exhaust in laboratory animals.
- Occupational exposure during coal gasification was determined by IARC (2010, 2012b) to be carcinogenic to humans based on consistent evidence for increased risk of lung cancer in studies of cohorts of coal gasification workers. Coal gasification workers are expected to be exposed to a wide range of chemicals including asbestos, silica, amines, numerous metals, aliphatic and aromatic hydrocarbons, sulfur dioxide, and aldehydes (IARC 2012b). In support of this determination, IARC also determined that there was: (1) sufficient evidence for the carcinogenicity of coal tars from gas works and manufactured gas plant residues in experimental

animals after dermal or oral exposure; and (2) strong evidence in experimental animals for a genotoxic carcinogenic mechanism involving mutagenic PAHs in coal gasification samples. The EPA IRIS (IRIS 2015) has not assessed the carcinogenicity of occupational exposure during coal gasification or manufactured gas residues.

The advantage of using toxicological or epidemiological data on the mixture of concern to determine a public health guidance value is that any interactions among the components of the mixture should be represented by the health effects data for the original mixture. Limitations of the use of original mixture data include the uncertainties regarding the extent to which the mixture of concern matches the mixture that is the basis for the health guidance value, due to changes in mixture composition with time and distance from the release, and/or differences in the original mixture released into the environment. Thus, for most exposure scenarios, the mixture of concern will likely not be identical to the mixture that is the basis for the health guidance value, even when it is called by the same name (e.g., toxaphene, commercial mixtures of PCBs).

3.2. SUFFICIENTLY SIMILAR MIXTURE APPROACH

If no adequate data are available on the mixture of concern, but health effects data or guidance values are available on a sufficiently similar mixture, the health hazard assessment may be based on the health effects data for the sufficiently similar mixture (see Chapter 2; EPA 1986, 2000, 2003).

Sufficiently similar mixtures are those having the same chemicals but in different proportions, or having most, but not all, chemicals in common and in similar proportions. In addition, sufficiently similar mixtures and their components have similar fate, transport, and health effects, whereas dissimilar mixtures do not.

ATSDR recommends the qualitative approach used by EPA (2000) in determining (or providing support for an assumption of) sufficient similarity. The approach considers the following criteria:

1. establish that common effects or common effects mediated by a common MOA are caused by short- or long-term exposure to the mixtures or their principal components;
2. identify common components across the mixtures in similar proportions;
3. establish a common source or process of formation across the mixtures; and

4. consider the results of time-dependent transformations of mixtures introduced into the environment.

3.2.1. Examples of Sufficient Similarity Approaches used for Hazard Identification and Dose-response Assessments

Discussion follows of several examples of mixtures that have been variously (not systematically) evaluated for sufficient similarity for hazard identification and dose-response assessment purposes: jet fuels (ATSDR 2015), gasolines (ASTM 2015; ATSDR 1995a, 1999; MassDEP 2002; Ohio EPA 2010; Oklahoma DEQ 2012; Total Petroleum Hydrocarbon Criteria Working Group 1997, 1998a, 1998b; Weisman 1998), PCB mixtures (ATSDR 2000b; EPA 1996; IARC 2015), and comparative potency method for combustion products (Albert et al. 1983; Lewtas 1985, 1988).

Jet fuels (JP-5, JP-8, and Jet A) are kerosene-based fuels refined from crude or shale-derived oil by straight or catalyst-assisted distillation (ATSDR 2015). Jet fuels are generally refined under more stringent performance-related conditions than kerosene and contain >200 aliphatic and aromatic hydrocarbons (C₆–C₁₇) as principal components, as well as additives (such as antioxidants, corrosion inhibitors, and biocides) that can vary from one fuel type to the other (ATSDR 2015). In 1998, ATSDR derived an intermediate-duration inhalation MRL for both JP-5 and JP-8 based on a study identifying liver effects in rats exposed by inhalation to JP-5 vapor (ATSDR 1998a). Although a formal consideration of sufficient similarity was not conducted in 1998, the recommendation to use the JP-5-based MRL for JP-8 exposures was based on a sufficient similarity assumption. ATSDR derived updated MRLs for jet fuels in 2013 using more recent animal toxicology studies indicating that jet fuels may not produce common critical effects (ATSDR 2015). ATSDR derived separate intermediate-duration inhalation MRLs for JP-5 (based on liver effects in rats) and JP-8 (based on neurotoxic effects in rats) and did not derive one for Jet A due to inadequate data. For oral exposures, ATSDR derived acute- and intermediate-duration oral MRLs for JP-8 (based on immunotoxic effects in mice), but no oral MRLs for JP-5 or Jet A due to inadequate data. The 2015 ATSDR Toxicological Profile did not directly discuss the possible utilization of MRLs based on data for one jet fuel type as surrogates (i.e., sufficiently similar mixtures) for jet fuels with inadequate data (ATSDR 2015).

ATSDR did not derive MRLs for automotive gasoline because of the wide compositional range of formulations for gasoline and the likelihood that components have widely differing environmental fate and transport properties (ATSDR 1995a, 1999; Pohl et al. 1997). This decision represented an application of several of the recommended qualitative determination criteria listed above. Consequently, exposed populations are likely to be exposed to fractions that are not sufficiently similar to the original

mixture. Parallel to this determination, other agencies have recommended modified component-based approaches to assessing risks from sites contaminated with gasoline and other petroleum products (e.g., ASTM 2015; MassDEP 2002; Ohio EPA 2010; Oklahoma DEQ 2012; Total Petroleum Hydrocarbon Criteria Working Group 1997, 1998a, 1998b; Weisman 1998). These approaches involve separating the complex mixture into groups of chemicals with similar chemical structures (e.g., aromatic hydrocarbons with 5–9 carbons, aliphatic hydrocarbons with 5–8 carbons), selecting a representative chemical with adequate dose-response data to indicate hazard potential and dose-response relationship for each group (i.e., an indicator chemical), and using the guidance value of the indicator chemical coupled with exposure estimates for all members of the group in the subject mixture to estimate health risk from the group.

PCBs are a class of 209 aromatic congeners, each containing 1–10 chlorines attached to the core biphenyl molecule (ATSDR 2000b; IARC 2015). Commercial PCB mixtures, previously used as coolants in electrical capacitors and transformers, were mixtures of many PCB congeners of variable composition (ATSDR 2000b; IARC 2015). The composition of environmental PCB mixtures can be different from that of commercial mixtures, because rates of weathering and biotransformation vary across PCB congeners and environmental conditions (ATSDR 2000b; IARC 2015). Discussion follows of three examples of assessments made for PCB mixtures that are variably dependent on an assumption of sufficient similarity.

IARC (2015) determined that PCBs in general and dioxin-like PCBs (PCBs that produce toxic effects through aryl hydrocarbon receptor mediation) are carcinogenic to humans, based on sufficient evidence of carcinogenicity from >70 epidemiology studies of PCB mixture-exposed workers and studies of experimental animals exposed to individual PCB congeners, commercial PCB mixtures, or synthetic mixtures of various PCB congeners, including simulated environmental mixtures of PCB congeners. Based on mechanistic data, IARC (2015) determined that individual PCBs cause cancer through multiple mechanisms and that the carcinogenicity of PCB mixtures cannot be solely attributed to the dioxin-like PCBs.

ATSDR derived an intermediate-duration oral MRL for PCB mixtures based on a LOAEL for neurobehavioral changes in infant monkeys exposed to a simulated environmental PCB mixture containing 80% of the congeners typically found in human breast milk samples (i.e., a simulated environmental mixture) and a chronic-duration oral MRL based on a LOAEL for immunological effects in adult monkeys fed encapsulated doses of a commercial PCB mixture (Aroclor 1254) for 23–55 months

(ATSDR 2000b). ATSDR acknowledged the compositional variation among commercial and environmental PCB mixtures, but derived oral MRLs for PCB mixtures by evaluating all available toxicity studies on commercial and synthetic PCB mixtures and selecting the studies with the lowest LOAEL values as the basis of the MRLs. Inherent in this process are the assumptions that PCB mixtures are sufficiently similar for dose-response assessment purposes and basing the MRLs on the lowest LOAELs from studies of specific PCB mixtures (i.e., a simulated environmental mixture and Aroclor 1254) would be protective for PCB mixtures in general. In support of the latter assumption, ATSDR noted that the chronic MRL based on immunological effects in adult monkeys was similar to a chronic MRL based on an estimated NOAEL for the lack of developmental effects in a study of children of North Carolina women exposed to environmental PCB mixtures as indicated by PCB levels in breast milk samples (ATSDR 2000b).

In a cancer dose-response assessment for PCB mixtures, EPA (1996) recommended a tiered risk assessment approach that used different slope factors (based on cancer bioassays with different commercial PCB mixtures differing in chlorine content) for different exposure scenarios. Exposure scenarios were grouped in consideration of how environmental processes influence the distribution of PCB congeners in environmental media. This recommendation was based on several pieces of evidence, including: (1) environmental PCB mixtures are expected to present increased risk for cancer because the compositional range of PCB congeners in commercial mixtures overlaps with the range in environmental PCB mixtures; (2) higher chlorinated congeners tend to be more potent and more persistent in soils and sediments than lower chlorinated congeners; (3) PCB congeners found in water or air tend to be lower in chlorine content than congeners found in soil, sediment, and tissues of animal species high in the food chain; and (4) bioaccumulative, high-chlorine content PCB congeners appear to be more potent carcinogens than commercial Aroclor mixtures. Individual human oral cancer slope factors were derived from tumor incidence data from five 2-year bioassays with rats exposed to one of four Aroclor PCB mixtures (1016, 1242, 1254, and 1260) varying in percentage chlorine content (41, 42, 54, and 60%, respectively). The OSFs (95th upper confidence limits on slope in units of risk per mg/kg/day) were: 0.07 (Aroclor 1016); 0.4 (Aroclor 1242); 1.5 (Aroclor 1254); 0.5 (Aroclor 1260); and 2.2 (Aroclor 1260). A composite OSF of 2 per mg/kg/day was recommended for high risk and persistence exposures including food-chain exposure, sediment or soil ingestion, dust or aerosol inhalation, and early-life exposures by any pathway. A composite OSF of 0.4 per mg/kg/day was recommended for low risk and persistence exposures, including ingestion of water-soluble congeners, inhalation of evaporated congeners, and dermal exposures. The lowest slope factor of 0.07 per mg/kg-day was recommended for exposure to PCB mixtures containing PCB congeners with more than four chlorines, accounting for <0.05% of total PCBs.

The comparative potency method uses data for a set of similar mixtures to estimate a scaling factor that relates cancer potency derived from a chronic animal study or human epidemiology study to potency in a mouse skin painting assay. The cancer potency factor for an additional similar mixture for which only data from the skin painting assay are available can be estimated using this scaling factor (Calabrese 1991; EPA 2000; Hertzberg et al. 1999; NRC 1988). This method was used in the estimation of human cancer risk from very complex mixtures of combustion emissions from various sources (Albert et al. 1983; Lewtas 1985, 1988), but it has not been applied in site-specific public health or risk assessments.

3.2.2. Future Approaches to Sufficient Similarity Assessments

Recent methods have been proposed for determining sufficient similarity among mixtures of pyrethroid insecticides found in surface wipe samples from U.S. child care centers (Marshall et al. 2013) and mixtures of disinfection byproducts in drinking water (Feder et al. 2009a, 2009b). These methods require additional review across expert panels and regulatory and public health agencies before they are widely accepted.

Marshall et al. (2013) developed a statistical method for assessing sufficient similarity of mixtures of up to 15 pyrethroid insecticides detected in floor wipe samples collected in 2001 from multiple locations in 168 U.S. licensed child care centers, relative to a reference mixture (of five pyrethroids) with dose-response data from an acute, oral exposure study of a neurological end point (motor activity) in rats (Wolansky et al. 2009). In this analysis, each floor-wipe sample was considered a unique mixture without any dose-response data. The composition and mixing ratio of the five-pyrethroid reference mixture was based on the average percent composition of the six most prevalent components in the floor-wipe samples with the top 10% highest total pyrethroid concentrations; the most prevalent components included cis- and trans-permethrin, which were combined into one-component (permethrins) in the five-component reference mixture. The method used to test whether or not any of the floor-wipe “mixtures” were sufficiently similar to the reference mixture was a modification of a statistical method described by Stork et al. (2008), which uses equivalence testing methodology comparing Euclidean distances between benchmark dose (BMD) estimates for different mixtures. Because the floor-wipe mixture did not have dose-response data to derive BMDs, a modification of the Stork et al. (2008) method was made, based on an assumption that BMDs for the “floor-wipe mixtures” could be estimated from the proportions of the analyzed 15 pyrethroids in them and the BMD for the reference mixture. Among the 168 floor-wipe samples, 42 had concentrations for each of the 15 pyrethroids that were below the detection limit. For the

remaining 126 floor-wipe samples, seven were determined to be sufficiently similar to the reference mixture using the modified method. In a subsequent analysis that adjusted the floor-wipe-mixture estimated BMDs and the BMD for the reference mixture by relative potency factors for the individual pyrethroids, 114/126 floor-wipe samples were determined to be sufficiently similar to the reference mixture.

Feder et al. (2009a, 2009b) applied multivariate statistical procedures to a data set describing chemical composition variables associated with disinfection processes and mutagenic activities of samples of finished water and distribution system water from five water treatment plants. The analysis included six chemical characteristics (total organic carbon, total organic halogens, total trihalomethanes, six haloacetic acids, percent brominated total trihalomethanes, and percent brominated haloacetic acids) and mutagenic activities of the samples. The statistical analysis indicated that the finished (post treatment) samples from the groundwater treatment plant were significantly different from the finished samples from the four surface water treatment plants, and that differences among the four surface water treatment plants were less clearly indicated. The four samples were sufficiently similar mixtures

Other future applications to predict high, medium, or low cancer potencies of very complex mixtures may involve predictions from analyses of gene expression profiles from short-term exposure (Tilton et al. 2015). Tilton et al. (2015) analyzed gene expression profiles in skin of mice collected 12 hours after applying tumor-initiating doses of individual PAHs (benzo[a]pyrene or dibenzo[def,p] chrysene) or very complex PAH-containing mixtures (diesel particulate extracts, coal tar extracts, or cigarette smoke condensate) and compared the results of the analysis with tumor outcomes. The analyses identified short-term-initiated biological signaling pathways that were predictive of tumor initiation potency classified as high, medium, or low (Tilton et al. 2015).

A more quantitative experimental basis to the determination of sufficient similarity among complex mixtures involves: (1) advanced chemical analytical capabilities (e.g., gas chromatography [GC]/mass spectrometry [MS], GC/flame ionization detection, and 2-dimensional GC); (2) statistical techniques for pattern recognition and principal component analysis; and (3) multivariate regression techniques to link chemical components with activity in biological tests (Eide et al. 2002, 2004; Feder et al. 2009a, 2009b; Teuschler 2007; Tian et al. 2015; Ventura et al. 2011). For example, Eide et al. (2002, 2004) used principal component analysis to analyze GC/MS data for 20–33 samples of organic extracts of exhaust particles collected from diesel engines, gas-fired furnaces, or fuel oil furnaces, followed by multivariate regression analysis to correlate the compositional data with measured activities of the samples in the

Ames mutagenicity test. A regression model was developed that identified compounds in the mixtures that co-varied with biological activity. The regression model could be used to predict mutagenicity of another exhaust particle sample with GC/MS data and provide quantitative information to determine sufficient similarity of two or more exhaust particle samples. Tian et al. (2015) used a similar approach to relate *in vitro* Chinese hamster ovary cell cytotoxicity of 40 samples of complex mixtures of organic chemicals extracted from polluted water in Shenqiu County of the Huai River basin in China with compositional data from GC/MS. A regression model was constructed from training datasets from 32 of the 40 samples. The model was used to compare predicted and observed cytotoxicity values obtained from eight “test” samples. The model explained about 92% of the cytotoxicity variability in the training data set, but only about 40% in the test data sets. This result suggests an inadequacy of the model to explain the variability in the test data sets and indicates compositional or biological activity dissimilarities between the training and test data samples (Tian et al. 2015).

Another research effort has developed a series of statistical screening models to predict several types of toxic end points (general, developmental, reproductive, and genetic toxicity) based on the polycyclic aromatic compound contents of a class of complex petroleum-derived mixtures called high-boiling petroleum substances (HBPS) (Gray et al. 2013; McKee et al. 2013; Murray et al. 2013a, 2013b; Nicolich et al. 2013; Roth et al. 2013). HBPS are complex mixtures typically composed of thousands of chemicals with final boiling points $\geq 650^{\circ}\text{F}$ (Gray et al. 2013). HBPS include various substances from petroleum refining streams called asphalts, aromatic extracts, crude oils, gas oils, heavy fuel oils, lubricating oil basestock, waxes, and residual hydrocarbon wastes (Gray et al. 2013). The composition of HBPS (even those with the same name) can vary due to compositional variations in crude oil starting materials, refining conditions, and product specifications (Gray et al. 2013). In a seminal research report examining systemic and developmental effects following repeated dermal exposure of rats to a number of HBPS (gas oils, heavy fuel oil components, and distillate aromatic extracts), common outcomes included increased liver weight, decreased thymus weight, decreased blood end points, increased resorption frequency, and decreased fetal weight (Feuston et al. 1994). The lowest-observed-effect levels in these studies were correlated (Spearman rank test) with the polycyclic aromatic compound weight percent of dimethyl sulfoxide (DMSO) extracts of the test HBPS samples, providing evidence that the polycyclic aromatic compound components were related to the effects of the original mixtures. Subsequent efforts used data from 39 dermal toxicity studies of HBPS samples with polycyclic aromatic compound compositional data to develop predictive models for repeated dose and developmental toxicity end points (Murray et al. 2013a, 2013b; Nicolich et al. 2013; Roth et al. 2013) and data from bacterial mutagenicity assays from 193 samples from several types of HBPS to develop predictive models for mutagenicity end points

(McKee et al. 2013). The statistical correlation models linearly regressed four repeated-dose end points (absolute thymus weight, hemoglobin count, platelet count, and relative liver weight) and three developmental end points (liver fetus count, fetal weight, and percent resorptions) against polycyclic aromatic compound contents (and other explanatory variables) (Nicolich et al. 2013). Several analytical measures of polycyclic aromatic compound contents were investigated as explanatory variables for the models, but the best fits were obtained with weight-percent of DMSO-extracts in seven aromatic ring classes (1, 2, 3, 4, 5, 6, and 7 and more rings) and other biological variables (such as body weight, gender, and duration of exposure for the systemic repeated dose end points and control group values for the developmental end points) (Murray et al. 2013a; Roth et al. 2013). Correlations (“r values”) between observed doses associated with specific responses and values predicted with the final models were >0.9 (Nicolich et al. 2013). The authors suggested that the systemic and developmental toxicity models would be useful for screening untested complex mixture samples with polycyclic aromatic compound compositional data for setting priorities for further biological testing (Nicolich et al. 2013). They further noted that the data should not be used to draw conclusions about whether the polycyclic aromatic compound content is the cause of the repeated-dose or developmental toxicity, only that the polycyclic aromatic compound content of a petroleum substance may allow an estimate of toxicity through modeling. The statistical model developed to predict the general mutagenic outcome for HBPS samples in a modified *Salmonella* assay based on the polycyclic aromatic compound content of the samples predicted the mutagenic outcome of 99% of the 193 data sets used to develop the model and 94% of 49 data sets not used to develop the model (McKee et al. 2013). The general outcome used a concept termed the “mutagenicity index,” defined as the slope of the initial portion of the dose-response curve. The model predicted a final binary mutagenicity index outcome as either <1 or ≥ 1 .

3.2.3. Current Limitations to Sufficient Similarity Approaches

As discussed earlier in this chapter, determination of sufficient similarity between mixtures often requires qualitative judgement after evaluation of available information on the chemical composition and biological activities of any two or more mixtures. Chemical information alone may not be sufficient to have confidence in a determination of sufficient similarity for hazard identification or dose-response purposes, especially for complex mixtures like engine exhausts, wood preserving wastes, coal tars, or manufactured gas waste residues (Cizmas et al. 2004; DeMarini et al. 1989; Simmons and Berman 1989). Even with data on comparative biological activities, determination and validation of sufficient similarity among complex mixtures is challenging due to: (1) variable source and weathering conditions that can influence chemical composition; (2) unidentified and variable toxic chemicals in the mixtures; and (3)

unexpected or unknown interactions that may occur among mixture components (Cizmas et al. 2004; Eide et al. 2002, 2004; Rice et al. 2009; Teuschler 2007). For example, in a study of two wood preserving waste mixtures containing PAHs, pentachlorophenol, and other chemicals, the order of the observed activities of fractionated crude extracts of the mixtures in *in vitro* genotoxicity tests were not well correlated with the activities that were expected based on chemical composition and relative potencies of the known components (Cizmas et al. 2004).

3.3. COMPONENT-BASED APPROACHES

3.3.1. Issues Related to Component-Based Approaches

3.3.1.1 Concepts of Additivity

Due to the lack of suitable health criteria for the mixture of concern or a sufficiently similar mixture, approaches involving the components of a mixture are commonly used for mixtures associated with contaminated sites. These methods are based on an assumption that the exposures or the responses to the mixture components are additive. The classical statistical concepts of dose addition and response addition are based on assumptions of common or different modes or mechanisms of action, respectively (Bliss 1939; Finney 1971), whereas a more generalized dose-addition concept proposed by Berenbaum (1985) and Gennings et al. (2005) does not require assumptions about mechanisms of action.

Dose Addition. 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; Loewe and Muischnek 1926). 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 MOA (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, and the TEF and RPF approaches (Sections 3.3.2 and 3.3.5).

Response Addition. Response addition, also known as *simple independent action* and *independent joint action* (Bliss 1939), assumes that the chemicals act independently and by different MOAs. Three mathematical definitions of response addition have been described based on the direction and degree to which the distribution of tolerance (or susceptibility) to one component may or may not be correlated with the distribution of tolerance to another component. When tolerances are completely positively correlated ($r=+1$), the order of individual tolerances to chemical A are identical to that of individual tolerances to chemical B. When the tolerances are completely negatively correlated ($r=-1$), the orders of individual tolerances to chemicals A and B are directly opposite. The third condition for the mathematical description of response addition is when there is no correlation ($r=0$) between the order of individual tolerances to chemicals A and B. The response-additive equations estimate the response to a mixture from the probabilities of response to the individual components and the conditional correlation of tolerances. Response addition is the underlying assumption of: (1) an approach to cancer risk assessment for components of mixtures at Superfund sites (EPA 1989a); (2) EPA's (EPA 2000) and ATSDR's default screening-level approach to noncancer health assessment for components with dissimilar toxicity targets, when whole-mixture data and interaction data are not available and exposure levels for components are below guidance values (RfCs, RfDs, or MRLs); and (3) the American Conference of Governmental Industrial Hygienists (ACGIH) approach to assessing the hazard of occupational exposure to agents that act independently (see Section 3.3.6 and Appendix C, Section C.1).

Generalized Dose Addition. Berenbaum (1985) described a general definition of additivity, which does not require chemicals in a mixture to have a common mechanism of action and which Gennings et al. (2005) algebraically related to statistical additivity models to be used in a method for assessing toxicological interactions in mixtures. In the statistical additivity models, when the rate of change in response of a chemical in a mixture (i.e., the slope of the dose-response relationship) does not change in response to other chemicals in the mixture, the chemical is claimed to act additively with the other chemicals (i.e., no interaction occurs). When the slope changes, an interaction (a deviation from additivity) is claimed. The method described by Gennings et al. (2002, 2004, 2005) requires descriptive dose-response data for each individual chemical in the mixture, as well as dose-response data for the mixture, but does not require the assumption of common mechanism of action or common adverse outcome for mixture components. This method was used to determine whether or not deviations from additivity occurred in responses of serum thyroxine levels in rats given four daily gavage doses of an 18-component mixture of polyhalogenated aromatic compounds at six dose levels (Crofton et al. 2005).

On theoretical grounds, Bosgra et al. (2009) questioned the general applicability of Berenbaum's general definition of additivity and provided an example of a biochemical mechanism in which two chemicals do not interact, but for which methods based on Berenbaum's definition would predict interaction. Bosgra et al. (2009) recognized the pragmatic usefulness of statistical methods based on Berenbaum's general definition in empirically assessing joint toxic action of chemicals in a mixture, but warned that deviations from additivity with these methods are incapable of defining specific biochemical mechanisms of interaction.

Dose Addition as a Public Health Protective Action. An underlying public health protective impetus of recommendations for screening level dose additivity is demonstrated by considering exposure to two theoretical chemicals, A and B, at exposure levels (0.9 units for A and 9 units for B), slightly below their respective toxicity guidance values (such as MRLs or RfDs) of 1 and 10 units (see also Appendix A for further illustrations). Response addition for the condition of completely negative correlations between susceptibilities to A and B predicts that exposure to A and B at these subtoxic levels would not produce an adverse effect [(response to A + B) = (response to A = 0) + (response to B = 0) = 0], but dose addition, using the concept of adding HQs (the exposure level to an agent divided by its toxicity guidance value), would indicate a concern for health hazard (hazard index = HQ A + HQ B = (0.9/1) + (9/10) = 0.9 + 0.9 = 1.8; see next section for further discussion of the hazard index approach, where HQs and hazard indices <1 indicate no hazard, and HQs and hazard indices >1 indicate increased risk for hazard).

Additional detail regarding dose addition and response addition is provided in Appendix A.

3.3.1.2 Evidence to Support or Refute the Use of Default Dose-Additivity Approaches

Until 1991, most published toxicological studies of possible interactions among environmental chemicals involved only pairs of chemicals (Hertzberg and Teuschler 2002; Krishnan and Brodeur 1991). Although the principles for statistically assessing deviations from additivity (either dose addition or response addition) had long been laid out in the published literature (e.g., Bliss 1939; Loewe and Muischnek 1926), many published toxicological studies on binary mixtures of environmental chemicals or drugs claiming to provide evidence for synergy or antagonism were inadequately designed to support the claims (Berenbaum 1989, 1990; Boobis et al. 2011; Borgert et al. 2001; Hertzberg and Teuschler 2002; Krishnan and Brodeur 1991). Most of the studies lacked suitable designs to conduct formal statistical tests to determine whether the responses to the mixture were different from the "no-interaction" hypotheses of dose additivity or response additivity. However, several early studies using overtly toxic acute doses of

binary mixtures showed that deviations from dose additivity were generally less than a factor of 5 (e.g., Smyth et al. 1969, 1970; Withey and Hall 1975; see Appendix A for more discussion). Likewise, toxicity studies on guppies and frogs using mixtures of 3 to as many as 50 components also tended to indicate that deviations from dose addition were not substantial (e.g., Dawson 1994; Hermens et al. 1985; Konemann 1981). Other studies were designed to test whether or not adverse effects could be observed when components of four- to nine-component mixtures were at NOAELs, but these studies lacked suitable designs to conduct formal statistical tests to determine whether the responses to the mixture were different from the “no-interaction” hypotheses of dose additivity or response additivity. Dose-additivity appeared to adequately describe the toxic action of a mixture of four kidney toxicants with a common MOA in rats fed a mixture of the four components in food for 4 weeks (Feron et al. 1995), whereas less-than-dose additivity or response additivity appeared to adequately describe toxic actions of mixtures of eight (Jonker et al. 1990) or nine (Groten et al. 1997) chemicals with dissimilar MOAs and targets, or a mixture of four kidney toxicants with dissimilar MOAs (Jonker et al. 1993). These types of observations have been used to support recommendations to use screening-level, component-based, dose-addition approaches for mixtures of chemicals having common MOAs or common adverse outcomes or target organs (e.g., this framework and ACGIH 2015; EPA 1986, 2000, 2002b, 2003; Meek et al. 2011, NRC 2004b). For mixtures of chemicals not having common MOAs or common adverse outcomes or target organs, some organizations recommend screening-level, component-based approaches based on response addition (i.e., independent action) (ACGIH 2015; EPA 2000).

Results from in vivo Studies. A number of animal studies conducted after the seminal review by Krishnan and Brodeur (1991) have used adequate designs to examine whether or not dose addition or response addition provided adequate descriptions of dose-response data for defined mixtures of more than two environmental chemicals having common MOAs or common adverse outcomes and to determine whether or not there were interactions among the components (Borgert et al. 2012; Cao et al. 2011; Christiansen et al. 2009; Crofton et al. 2005; EPA 2006b, 2007b, 2011b; Fattore et al. 2000; Gao et al. 1999; Gennings et al. 2002; Hamm et al. 2003; Hass et al. 2007; Hertzberg et al. 2013; Howdeshell et al. 2008; Jarvis et al. 2014; Moser et al. 2005, 2006, 2012; Nesnow et al. 1998; NRC 2008; Padilla 2006; Rider et al. 2008; Starr et al. 2012; Tajima et al. 2002; Van den Berg et al. 2006; Walker et al. 2005; Wolansky et al. 2009). In the context of these studies (summaries of results follow) and this framework, interactions among components of a mixture are defined as deviations from what would be expected if there were no interactions. If dose addition is the expected “no interaction” model, then observations greater than responses predicted by dose additivity are synonymous with synergy and observations less than predicted responses are synonymous with antagonism.

- Greater-than-response additive effects (synergy) at lower doses and less-than-response additive effects (antagonism) at higher doses were observed in lung tumor responses in mice exposed to single intraperitoneal injections of two dose levels of a mixture of five nonsubstituted PAHs at ratios similar to ratios in environmental air and combustion samples (Nesnow et al. 1998). The experimental design was a 2^5 factorial, 32-dose group scheme yielding lung adenoma per mouse data (8 months after dose administration). A response surface model based on response addition was used to predict lung tumor responses to compare with observed responses for each of the 32 “quintary” dose groups. Deviations from response additivity were small and less than about 2-fold different from the response additivity predictions. Earlier *in vivo* and *in vitro* studies of binary combinations of PAHs in producing cancer or cancer-related effects provided conflicting evidence for both greater-than-additive and less-than-additive interactions, depending on the evaluated compounds, examined end points, and test system, although most of these studies were not adequately designed to statistically test for consistency with dose additivity or response additivity (Jarvis et al. 2014; Nesnow et al. 1998). Possible contributing factors to deviations from additivity have been proposed, such as competitive inhibition or differential induction of bioactivating or detoxifying enzymes, but definitive conclusions about the underlying mechanisms of biochemical interactions among PAHs cannot be drawn because of the complexity of bioactivation and detoxification mechanisms and the complexity of the development of cancer (Jarvis et al. 2014; Nesnow et al. 1998).
- Greater-than-dose-additive effects were observed on several neurological end points in adult or weanling rats orally exposed to mixtures of five or four organophosphorus insecticides at relative proportions similar to those observed in the U.S. diet (Moser et al. 2005, 2006; Padilla 2006). Comparison of predicted (using a dose-additive model based on dose-response relationships for the individual pesticides) and empirical ED₂₀ and ED₅₀ values for the end points indicated that the greater-than-additive effects were small, from about 1.2–3-fold in magnitude. Earlier studies of the lethality of 43 pairs of organophosphorus insecticides in rats indicated that dose additivity explained 21 pairs, 18 pairs showed less-than-additive effects, and only 4 pairs showed greater-than-additive effects (Dubois 1961). The EPA (2006b) cumulative risk assessment for organophosphorus insecticides concluded that dose addition is a reasonable approach for estimating cumulative risk of mixtures of organophosphorus insecticides, and that the available data did not provide a sufficient basis to depart from dose additivity, based on: (1) these data; (2) other data indicating that toxicokinetic interactions between organophosphorus insecticides

can be complex; and (3) evidence that organophosphorus insecticides have a common mechanism of action (cholinesterase inhibition) in producing neurological effects.

- Dose additivity adequately explained adverse neurological end points (e.g., brain cholinesterase activity and motor activity) in adult rats given single oral doses at five levels of a seven-component mixture of N-methyl carbamates (EPA 2007b). The mixture was designed to deliver equipotent contributions by the components to brain cholinesterase inhibition based on dose-response relationships characterized for each component alone. Ninety-five percent confidence intervals for brain cholinesterase activities predicted by dose additivity overlapped with observed values. The EPA (2007b) cumulative risk assessment for N-methyl carbamates concluded that dose additivity for cumulative risk assessment of mixtures of these pesticides is “reasonable” for this group of insecticides representing a common mechanism of action group (cholinesterase inhibition by a different mechanism than organophosphorus insecticides). An expanded study examined motor activity and cholinesterase activities (brain and red blood cells) in adult and weanling rats given single oral doses of five levels of the seven-component equipotent mixture or another environmentally relevant mixture containing the same components in a different mixing ratio based on California sales data for these pesticides (Moser et al. 2012). Using a statistical approach described by Hertzberg et al. (2013), the equipotent mixture results for adult rats showed dose additivity for red blood cell cholinesterase and motor activity and greater-than-dose additivity for brain cholinesterase at a middle dose level only; for weanling rats, brain cholinesterase and motor activity were dose additive and red blood cell cholinesterase was slightly less-than-dose additive. Exposure of both ages to the other mixture showed greater than dose additivity (synergy) on all three end points, but the magnitude of deviation from dose additivity was small, ranging from 1.5- to 2.6-fold for the different end points in the two ages of rat (Moser et al. 2012). The results also indicate that interactions (i.e., deviations from dose additivity) can be dependent on end point examined, age or stage of development, and mixing ratios of components.
- Dose additivity adequately explained the joint action of mixtures of 11 pyrethroid insecticides (Wolansky et al. 2009) or 5 pyrethroid insecticides (Starr et al. 2012) on motor activity in rats given single gavage doses of the mixtures. No statistically significant differences were found in observed motor activity values and predicted responses based on dose additivity and descriptions of the dose-response relationships for the individual components. A companion *in vitro* study assessing sodium influx in cerebrocortical neurons (presumably mediated by voltage-gated

sodium channels) found no statistically significant differences between observed effects from the 11-component mixture and effects predicted by a dose-additivity model (Cao et al. 2011). Based on results from these studies, the EPA (2011b) concluded that dose addition is a reasonable approach for estimating cumulative risk of exposures to mixtures of pyrethroids, noting several areas of uncertainty associated with this conclusion including whether dose additivity would predict responses to mixtures with different mixing ratios of components or by different exposure routes and duration.

- Statistically significant, greater-than-dose-additive effects at high doses and no significant deviation from dose additivity at low doses were observed on serum total thyroxine levels in young female rats given four daily gavage doses of an 18-component mixture of polyhalogenated aromatic hydrocarbons (2 dioxins, 4 dibenzofurans, and 12 PCBs, including dioxin-like and non-dioxin-like PCBs) (Crofton et al. 2005). The mixing ratio of the components was based on ratios of these chemicals found in breast milk, fish, and other sources of human exposure, and the mixture was given to rats at six dose levels ranging from approximately background levels to 100-fold greater than human background levels. The study included six to nine dose groups for each component to adequately characterize individual dose-response relationships. The statistical analysis used methods described by Gennings et al. (2002, 2004) and the definition of additivity described by Berenbaum (1985). Predicted responses based on additivity were about 2–3-fold less than observed responses at the three highest dose levels of the mixture, indicating a dose-dependent, greater-than-dose additive joint toxic action of relatively small magnitude.
- In several studies examining male reproductive system developmental end points (e.g., anogenital distance, nipple retention, testosterone production, other reproductive tissue malformations) in offspring of rats orally exposed to mixtures of chemicals that variably produce anti-androgenic effects via different mechanisms, dose-additive models provided adequate predictions of observed effects for most of the studies. The studied mixtures included vinclozolin, flutamide, and procymidone (Hass et al. 2007); diethylhexyl phthalate, vinclozolin, prochloraz, and fiansteride (Christiansen et al. 2009); di(*n*)butyl phthalate and diethylhexyl phthalate (Howdeshell et al. 2007); butyl benzyl phthalate, diethylhexyl phthalate, di(*n*)butyl phthalate, diisobutyl phthalate, and dipentyl phthalate (Howdeshell et al. 2008); and vinclozolin, procymidone, prochloraz, linuron, butyl benzyl phthalate, diethylhexyl phthalate, and di(*n*)butyl phthalate (Rider et al. 2008). Based on the results in these studies, the National Research Council (NRC) report, *Phthalates and Cumulative Risk Assessment: Tasks Ahead* (NRC 2008), recommended

that cumulative risk assessments should be conducted for phthalates producing common adverse outcomes on the developing male reproductive system using a dose-additive approach, regardless of mechanisms of action. Borgert et al. (2012) presented a critical evaluation of this recommendation noting several areas of uncertainty including limitations of the supporting study designs and analyses (e.g., each of the studies only looked at one mixing ratio of the components), extrapolations from relatively high exposure levels used in the rat studies to lower exposure levels expected to be experienced by humans, and evidence that humans may be less sensitive than rats to anti-androgenic chemicals, and commenting that a dose-additive/common adverse outcome approach to cumulative risk for phthalates and other anti-androgenic agents should only be used as a coarse, screening-level assessment.

- The TEF approach to assessing risks from mixtures of chlorinated dibenzo dioxins (CDDs) and related compounds is based on the assumption of dose additivity (see Section 3.3.5 for more details of this approach). Results from *in vivo* studies of animals exposed to defined mixtures of dioxins and dioxin-like compounds indicated that World Health Organization (WHO) recommended TEF values (Van den Berg et al. 2006) predicted mixture toxicities within a factor of about 2 or less (Fattore et al. 2000; Gao et al. 1999; Hamm et al. 2003; Walker et al. 2005), providing evidence that the dose additivity assumption in the TEF approach for dioxins and dioxin-like compounds is useful.

Results from in vitro Studies. Results from adequately designed *in vitro* studies of several end points (e.g., androgen receptor (AR) antagonism, estrogen receptor (ER) activation, ER-mediated cell proliferation, several genotoxicity end points) in cultured cells exposed to synthetic mixtures of up to 20–30 chemicals provide similar evidence that deviations from concentration addition (i.e., dose addition), when found, were small from a risk assessment perspective (mostly <5-fold deviation).

Androgen Receptor Antagonism Studies

- Concentration addition provided reasonable predictions of anti-androgenic activity (AR antagonism) in cultured human breast cells (MDA-kb2) exposed to mixtures of 8 pesticides showing only AR antagonism (Orton et al. 2012) and 17 AR antagonists with varying structural features (Ermler et al. 2011). The reporter gene assay used in these studies measured luciferase induction after AR activation by binding of an AR agonist (alpha-dihydrotestosterone, DHT); AR antagonism was measured in terms of suppression of DHT-induced luciferase-mediated

luminescence. The eight pesticides with only AR antagonism were fludioxonil, fenhexamid, ortho-phenyl phenol, imazalil, tebucoazole, diethomorph, methiocarb, and primiphos-methyl. The 17 AR antagonists included several parabens (e.g., n-butyl paraben), UV-filter substances, benzo[a]pyrene, antioxidants (e.g., butylated hydroxytoluol), perfluorinated compounds, polybrominated and polychlorinated biphenyl ethers, and bisphenol A.

- Statistically significant deviations from concentration addition predictions were observed with mixtures of 5 pesticides, each showing both AR antagonism and agonism activities (cypronil, pyrimethanil, viclosolin, chlorpropham, and linuron tested at two mixing ratios) or mixtures of 13 pesticides (8 AR antagonists only and 5 with mixed antagonism and agonism activities tested at four mixing ratios), but these deviations were generally not large (Orton et al. 2012). Values of predicted ICs (IC_{10} or IC_{50}) for anti-androgenicity that were statistically significantly different from observed values ($n=6$) were mostly within 2-fold of observed values: five were lower than observed values but within 2-fold and one predicted value was greater than the observed value by about 6-fold (Orton et al. 2012).
- In another study with the same reporter gene assay, MDA-kb2 cells were exposed to mixtures of 30 AR antagonists from various classes of chemicals at three mixing ratios (Orton et al. 2014). Chemicals included pesticides, antioxidants, parabens, UV-filters, synthetic musks, bisphenol A, benzo[a]pyrene, perfluorooctane sulfonate, and pentabromodiphenyl ether. IC values predicted by concentration addition were slightly lower than observed values (within 2-fold) at all mixing ratios, whereas IC values predicted by independent action were greater than observed values by 2–4-fold (Orton et al. 2014).
- Concentration addition provided reasonable predictions of AR antagonism in an AR-reporter gene assay with Chinese hamster ovary CHO-K1 cells exposed to an equimolar mixture of two azole fungicides and one dithiocarbamate fungicide (biteranol, propiconazole, and mancozeb), but underestimated observed responses to an equimolar mixture of one triazine herbicide, two azole fungicides, one pyrethroid insecticide, and one organophosphate insecticide (terbuthylazine, biteranol, propiconazole, cypermethrin, and malathion) (Kjeldsen et al. 2013). For the five-component mixture, concentration addition predicted IC_{70} , IC_{80} , and IC_{90} values that were about 3-, 4-, and 9-fold higher than observed values (indicating greater-than-additive joint action).

- Another study reported that concentration addition adequately predicted the AR-antagonistic response in transfected CHO-K1 cells exposed to an equimolar mixture of five dissimilarly acting pesticides, deltamethrin (a pyrethroid insecticide), methiocarb (a carbamate insecticide), prochloraz (an azole fungicide), tribenuron-methyl (a sulfonyleurea herbicide), and simazine (a triazine herbicide) (Birkhøj et al. 2004).

ER Activation or ER-Mediated Cell Proliferation Studies

- Concentration addition provided reasonable predictions of ER activation, with some reports of small deviations from additivity, in reporter gene assays of transfected mammalian cells or yeast cells exposed to mixtures of up to 3–17 estrogenic chemicals (Charles et al. 2002a, 2002b, 2007; Evans et al. 2012; Le Page et al. 2006; Payne et al. 2000; Rajapakse et al. 2002; Silva et al. 2002), as well as in cell proliferation assays in ER-competent MCF-7 human breast cancer cells exposed to mixtures of up to 17 estrogenic chemicals (Evans et al. 2012; Payne et al. 2001; Rajapakse et al. 2004; Silva et al. 2011; van Meeuwen et al. 2007). Results from a sample of these studies follows.
 - ER activation in transfected human MCF-7 breast cancer cells exposed to a fixed-ratio mixture of six synthetic chemicals with estrogenic activity (methoxychlor, o,p-DDT, octylphenol, bisphenol A, β -hexachlorocyclohexane, and 2,3-bis-(4-hydroxyphenyl)-propionitrile) was less than additive across a range of concentrations, but the magnitudes of deviation from concentration addition at each of the tested concentrations were <3-fold (Charles et al. 2007). In a companion *in vivo* immature rat uterotrophic assay, observed uterine weight responses to a mixture of these chemicals were statistically consistent with dose addition (Charles et al. 2007).
 - Responses to mixtures of 13–17 estrogenic chemicals at various mixing ratios were generally consistent with concentration addition in an ER reporter gene assay with human T47D-KBluc breast cancer cells and in a cell proliferation assay with MCF-7 cells (Evans et al. 2012). Low concentrations in the nanomolar range of a mixture of 16 chemicals (each component with minimal estrogenicity alone) did not affect the cell proliferative response of MCF-7 cells to a 14-component mixture of estrogenic chemicals, but inhibited the response at concentrations in the micromolar range.

- Cell proliferation responses in MCF-7 cells exposed to mixtures of six phytoestrogens (coumestrol, genistein, naringenin, catechin, epicatechin, and quercetin), six synthetic chemicals with estrogenic activity (4-nonylphenol, octylphenol, β -hexachlorohexane, bisphenol A, methoxychlor, and dibutyl phthalate), or a combination of both mixtures showed no statistically significant deviations from concentration addition predictions (van Meeuwen et al. 2007).
- Mixtures of 8, 10, 11, or 16 estrogenic chemicals produced cell proliferative responses in MCF-7 cells that were: (1) adequately predicted by concentration addition for the 8-component mixture and (2) overestimated by concentration addition for the 10-, 11-, and 16-component mixtures, indicative of less-than-additive joint action (Silva et al. 2011). For the latter three mixtures, observed effective concentrations were greater than predicted values by factors ranging from about 1.5- to 5-fold (Silva et al. 2011).

Genotoxic End Point Studies

- Concentration addition adequately explained effects on micronuclei formation in CHO-K1 cells exposed to a mixture of seven aneugenic benzimidazole pesticides, which act by a similar mechanism: inhibition of microtubule formation by binding to β -tubulin monomers at the colchicine-binding site (Ermler et al. 2013). In a subsequent study of mixtures of four to five chemicals inducing micronuclei by different mechanisms (aneugens and clastogens), the observed micronuclei responses to the mixtures were larger than responses predicted by independent action (i.e., response addition), but less than those predicted by concentration addition (Ermler et al. 2014).
- Studies with cultured cells of mouse lymphoma cells (L5178Y) and human cell lines (TK 6 and WTK1) exposed to gamma-ionizing radiation from ^{137}Cs and ethyl methanesulfonate, showed micronuclei induction responses in the human cell lines that were consistent with concentration addition, but greater-than-additive action (40% supra-additive effect) in L5178Y cells (Lutz et al. 2002).
- Concentration addition predictions were not significantly different from observed mutation responses in bacteria (*Salmonella* Ames assay) to a mixture of three PAHs (benzo[a]pyrene, benz[a]anthracene, and dibenz[a,c]anthracene) (Lutz et al. 2002).

- Concentration addition predictions were mostly greater than observed mutation responses in several strains of *Salmonella* to complex mixtures that were nonpolar fractions of extracts from 10 soils contaminated with complex PAH-containing mixtures (Lemieux et al. 2008). The soils were from Swedish sites at which creosote wood preservation (n=7), coke production (n=1), or gas manufacturing (n=2) had occurred for many years. The concentration addition predictions were made based on individual relative potencies for eight nonsubstituted, homocyclic PAHs showing positive activity in the assay conducted with three *Salmonella typhimurium* strains. (Sixteen PAHs identified by the EPA as Priority PAHs were tested, and 8 showed positive results.) Sixty-eight percent of predicted values were statistically significantly greater than observed values; 94% of these values were within about 4-fold of observed values, and one was greater by about 8-fold (Lemieux et al. 2008). Twelve percent of predicted values were not significantly different from observed values, and 20% were less than corresponding observed values (Lemieux et al. 2008). In a subsequent study of mutagenic activities of nonpolar fractions of extracts of the same soils in an *in vitro* version of the LacZ transgenic rodent mutation assay, predicted values of mutagenic activity based on dose addition of individual mutagenic PAHs were within 2-fold of observed values for 9 out of 10 of the nonpolar fractions of soil extracts (Lemieux et al. 2015).
- In a tiered experimental design, deviations from response additivity, both greater-than-additive and less-than-additive, were detected in studies of the effects of mixtures of five mycotoxins with different mechanisms of action on inhibition of DNA synthesis in mouse fibroblast L929 cells (Tajima et al. 2002).

Summary of Evidence Related to Dose Additivity as a Default Assumption for Component-Based

Approaches to Assessing Noncancer Health Impacts. Based on the above *in vivo* and *in vitro* evidence, the dose-additivity assumption appears to be a reasonable *default* assumption for screening-level assessments of mixtures of chemicals with similar effects or the same target organ. Results from adequately designed studies of various end points affected by defined mixtures of various classes of chemicals showed that: (1) dose additivity often provided adequate descriptions of the mixture responses and (2) positive and negative deviations from dose additivity were small from a risk assessment perspective (generally <5-fold). In addition, results from a few studies of end points in cells exposed to mixtures of components with differing MOAs indicated that observed responses were intermediate between the values predicted by concentration addition (i.e., dose addition) and response addition (independent action) (Ermler et al. 2014; Orton et al. 2014).

In support of these conclusions, an independent analysis of research studies (published between 1990 and 2008) reporting “synergy” at dose levels close to PODs for individual mixture components (i.e., “low” doses) identified only 11 out of 90 studies reporting “synergy” in which the magnitude of “synergy” was calculated (Boobis et al. 2011). Among those 11 studies, 6 studies used comparable methods to indicate that the magnitude of synergy was small from a risk assessment perspective, ranging from about 1.9- to 3.5-fold greater than additivity (Boobis et al. 2011). Three of the six studies (Crofton et al. 2005; Moser et al. 2005, 2006) were described in the bulleted items above.

Although the research results reviewed herein provide support for the use of dose-additivity as a default assumption in component-based approaches, they also provide evidence for cases of deviations from additivity. In addition, much of the evidence is based on short-term exposures. Further research may help to confirm or refute the validity of this assumption, in particular for chronic exposure scenarios and for early life exposures with possible later life health outcomes. As such, environmental scientists should be aware that currently recommended approaches to assess health impacts from combined exposure to multiple agents (as discussed in Chapter 2) are practical tools which could overestimate or underestimate actual health impacts.

3.3.2. Hazard Index Approach

The hazard index approach uses the assumption of dose additivity to assess the noncancer health effects of a mixture from the data on the components. The approach, or some modification of it, is used or recommended by a number of agencies (including ATSDR), especially as a tool for screening-level assessments (see Chapter 4 of this document: ACGIH 2015; CPSC 2014; DEPA 2009; EC 2012; EFSA 2013; EPA 1986, 1989a, 2000, 2011c; Feron et al. 2004; Meek 2013; Meek et al. 2011; Mumtaz et al. 1994a, 1997; NAS 1974; Norwegian Scientific Committee for Food Safety 2013; NRC 1989; OSHA 1993, 2001; Yu et al. 2010, 2013). In this approach, exposures or doses for the various components of a mixture of concern are compared with a defined level of exposure generally regarded as acceptable or safe (public health guidance value) by the agency performing the assessment. The defined levels could be ATSDR MRLs, EPA RfDs or RfCs, ACGIH threshold limit values (TLVs), or Occupational Safety and Health Administration (OSHA) permissible exposure limits (PELs). The general equation for the hazard index (*HI*) is:

$$HI = \frac{E_1}{DL_1} + \frac{E_2}{DL_2} + \dots + \frac{E_n}{DL_n} \quad (1)$$

In Equation 1, E_1 is the level of exposure to the first chemical in the mixture and DL_1 is some defined level of “safe” exposure to the first chemical, E_2 and DL_2 are the corresponding levels for chemical 2, and the summation can extend to any number of chemicals, signified by the n . Each chemical-specific ratio (e.g., E_1/DL_1) is called a hazard quotient (HQ). Therefore, the hazard index can be expressed as the sum of the HQs:

$$HI = \sum_{i=1}^n HQ_i \quad (2)$$

When the HQ for a single chemical exceeds unity, concern for the potential hazard of the chemical increases. Similarly, when the hazard index for a mixture exceeds unity, concern for the potential hazard of the mixture increases.

Separate hazard indices are usually estimated for each exposure pathway and exposure duration of concern. For a given duration, hazard indices can be summed across pertinent exposure pathways that affect the same receptor population, giving an indication of cumulative impact or risk from components in the mixture.

The obvious advantage of this method is its simplicity. Because it is based on the assumption of dose additivity, the hazard index method is most appropriately applied to components that cause the same effect by the same mechanism or mode of action. In practice, it may be applied to components with different target organs as a screening measure. The method is also frequently applied to components with the same critical target organ or critical effect (effect that is the basis for the MRL, RfD, or other health guideline), without regard to mechanism or mode of action. For Superfund risk assessments, strong evidence is required to indicate that two compounds producing adverse effects on the same organ system, although by different mechanisms, should not be treated as dose additive (EPA 1989a, 2000). See also the discussion in Section 3.3.1.2 (*Evidence to Support or Refute the Use of Default Dose-Additivity Approaches*).

The ATSDR (2005a) *Public Health Assessment Guidance Manual* notes that there is no evidence of additive toxicity from exposure to components of a mixture when individual chemicals are administered well below their individual apparent toxicity thresholds (Seed et al. 1995; Wade et al. 2002). It recommends that when the site-specific hazard index is <1.0 , “it is highly unlikely that significant

additive or toxic interactions would occur, so no further evaluation is necessary.” When the hazard index exceeds 1.0, further evaluation is recommended, specifically that the assessor should compare the estimated exposure level for each component to the NOAEL on which the MRL is based. These comparisons represent POD HQs, as opposed to “guidance value” HQs. ATSDR (2005a) recommends that if exposure to one or more of the components is within 1 order of magnitude of the guidance value NOAEL (0.1xNOAEL), the assessor should conduct more in-depth analysis such as calculating hazard indices for components with common adverse effects (i.e., target organ or tissue) or common adverse effects via a common MOA and qualitatively evaluating information on possible interactions among components. Furthermore, ATSDR (2005a) recommends that if estimated exposure levels of all components are less than one-tenth of the respective PODs (i.e., NOAEL, LOAEL, or BMDL), then significant additive or interactive effects are unlikely and further in-depth evaluation of potential health impacts from exposures to multiple chemicals at the site is unnecessary. However, ATSDR (2005a) also noted that assessors may proceed with further evaluations in some instances, such as when several or more components in the mixture produce the same health effect either by different or common MOAs, when there are concerns for sensitive populations in the community, when the PODs for the MRLs are uncertain, or for other reasons (see Chapter 2 of this document for more specific and extensive discussion of assessing health impacts from multiple agents).

The hazard index method does not take into account interactions among the components of the mixture, but methods to modify the method by incorporating data on possible interactions (deviations from additivity) among components are described in Section 3.3.3.

Additional information on the hazard index method is provided in EPA (1986, 1989a, 2000).

3.3.3. Target-organ Toxicity Dose (TTD) Modification to Hazard Index Approach

The TTD approach, which is a refinement of the hazard index approach, was devised in order to accommodate the assessment of mixtures whose components do not all have the same critical effect (i.e., the most sensitive effect providing the basis of the public health guidance value), but may produce toxic effects in common target organs dependent on exposure level. It takes into account the reality that most components of contaminated-site-related mixtures affect other target organs at doses higher than those that cause the critical effect of the guidance value. These other effects may vary from component to component and may be important in assessing the health effects of the mixture. EPA (1989a) suggested that separate hazard indices be estimated for all end points of concern, and that the RfD be used not only

in generating HQs for the critical effect of a component, but also in estimating HQs for effects that occur at higher exposure levels. As acknowledged by EPA (1989a) and demonstrated by Mumtaz et al. (1994a, 1997), this practice may overestimate the hazard for effects occurring at exposure levels higher than those associated with the critical effect. The use of TTDs was therefore suggested (Mumtaz and Colman 1993; Mumtaz et al. 1997). TTDs are developed for the chemicals that affect an end point at a dose higher than that for the critical effect for the same chemical. A TTD for each end point of concern is calculated using appropriate MRL (or RfD) methodology, and then used in estimating the end-point-specific HQs and hazard indices. The MRL (or RfD) is used for the critical effect for each chemical and the TTD is used for the other end points of concern for the chemical. When any of the end-point-specific hazard indices exceeds unity, concern for the potential hazard of the mixture increases.

The derivation of TTDs for use in assessment of the joint toxic action of chemical mixtures is analogous to the derivation of MRLs, and should follow the applicable portions of ATSDR MRL guidance (ATSDR 1996). TTDs are based on the other major characteristic effects of a chemical, which are known to occur at the same or higher exposure levels as the critical effects. Like the derivation of an MRL, the derivation of a TTD is not recommended for an end point that is affected only at the relatively high levels of exposure associated with severe effects. Because the purpose of TTD derivation is to support the estimation of end-point-specific hazard indices (Mumtaz et al. 1994a, 1997), TTD derivations should be performed for end points that are common to more than one component of a given mixture. In addition, end points identified as concerns in populations exposed to the mixture should be considered.

Like MRLs (or RfDs), TTDs are specific for route and exposure period. The TTD should be based on the highest NOAEL that does not exceed a LOAEL for the particular end point, as determined from the information in toxicological profiles, including the Levels of Significant Exposure tables. If such a NOAEL is not available, the TTD would be based on the lowest LOAEL for that end point; PODs for TTDs should be from a representative, high-quality study involving the route and exposure duration of concern. When data for the exposure duration of concern are not available, a TTD derived for one duration may sometimes be applicable for other duration(s) of the same route, if supported by the overall database. An additional uncertainty factor may be applied to account for uncertainty associated with duration extrapolation, based on scientific judgment. Dose adjustments and interspecies, intraspecies, and LOAEL-to-NOAEL extrapolation (i.e., uncertainty factors) should be performed and explained as for an MRL. When suitable data are available, and when appropriate, TTDs can also be derived using BMD PODs (Crump 1984, 1995; EPA 2012a; Gaylor et al. 1998) to define the BMDL, which is used in place of a NOAEL as the basis for TTD derivation, similar to the procedure for MRL derivation.

An illustrative example follows of the application of the TTD-modification of the hazard index to a hypothetical site-specific mixture of chemicals 1, 2, 3, and 4 to which intermediate-duration oral exposure is of concern. The intermediate oral MRLs are based on critical hepatic effects for chemicals 1 and 2, and critical renal and critical developmental effects, respectively, for chemicals 3 and 4. Each of these end points also is affected by at least one other mixture component for which it is not the critical effect. Other major effects in common for two or more of these chemicals for this route and duration include neurological and developmental effects. In addition, chemical 1 causes immunological effects and chemical 4 causes endocrine (adrenal) effects during intermediate oral exposure. At levels of exposure that cause high mortality, chemical 1 also causes hematological effects in rats. This information is summarized in Table 3.

Table 3. End Points Affected by Chemicals 1, 2, 3, and 4

End point	Affected by:			
	Chemical 1	Chemical 2	Chemical 3	Chemical 4
Hematological	With mortality	No	No	No
<i>Hepatic</i>	Yes—MRL	Yes—MRL	No	Yes
<i>Renal</i>	Yes	No	Yes—MRL	Yes
Endocrine (adrenal)	No	No	No	Yes
Immunological	Yes	No	No	No
<i>Neurological</i>	Yes	Yes	Yes	No
<i>Developmental</i>	Yes	Yes	Yes	Yes—MRL

MRL = Minimal Risk Level

The end points of concern chosen for TTD derivation, based on the critical effects of the chemicals and on other major effects in common for this set of chemicals, are hepatic, renal, neurological, and developmental effects. These end points are shown in bold italicized print in the table. Since adrenal and immunological effects each are caused by only one chemical, and are not the critical effects for any of the components of the mixture, the estimation of end-point-specific hazard indices is not needed for these end points, and TTDs are accordingly not developed. For a different mixture of chemicals that included chemical #1, the immunological end point may warrant TTD derivation if at least one other chemical in the mixture also causes this effect. Similar reasoning would apply for chemical #4 and adrenal effects. The hematological effects are not a suitable basis for TTD derivation for chemical #1 not only because they are caused by only one chemical, but also because they occurred only at levels of exposure that caused significant mortality.

For the purposes of illustration, a TTD for renal effects will be derived for chemical #1. The intermediate oral MRL for chemical #1 is 0.15 mg/kg/day based on a NOAEL of 15 mg/kg/day for hepatic effects in experimental animals given the chemical orally for an intermediate duration. The NOAEL was divided by an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variability) to estimate the MRL. The LOAEL for hepatic effects in the same study was 30 mg/kg/day. The NOAEL and LOAEL values for renal effects in this study were 30 and 45 mg/kg/day, respectively, and were the most reliable data for this effect. In addition, the NOAEL was the highest NOAEL for this effect. A TTD_{RENAL} of 0.3 mg/kg/day for chemical #1 is derived by dividing the $NOAEL_{RENAL}$ of 30 mg/kg/day by an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variability). Derivation of TTDs for the other effects would proceed in a similar manner.

Following derivation of the TTDs, end-point-specific hazard indices are calculated as follows:

$$\begin{aligned}
 (a) \quad HI_{HEPATIC} &= \frac{E_1}{MRL_1} + \frac{E_2}{MRL_2} + \frac{E_4}{TTD_{4HEPATIC}} \\
 (b) \quad HI_{RENAL} &= \frac{E_1}{TTD_{1RENAL}} + \frac{E_3}{MRL_3} + \frac{E_4}{TTD_{4RENAL}} \\
 (c) \quad HI_{NEURO} &= \frac{E_1}{TTD_{1NEURO}} + \frac{E_2}{TTD_{2NEURO}} + \frac{E_3}{TTD_{3NEURO}} \\
 (d) \quad HI_{DEV} &= \frac{E_1}{TTD_{1DEV}} + \frac{E_2}{TTD_{2DEV}} + \frac{E_3}{TTD_{3DEV}} + \frac{E_4}{MRL_4}
 \end{aligned} \tag{3}$$

where $HI_{ENDPOINT}$ is the hazard index for indicated end point (*HEPATIC*, *RENAL*, *NEURO* [neurological], *DEV* [developmental]), E_i is the exposure for the i^{th} chemical (1, 2, 3, or 4 in the above example), MRL_i is the MRL for the i^{th} chemical, and TTD_i is the TTD for the i^{th} chemical for the indicated end point. (If an MRL is not available, a suitable RfD can be used.) Although developmental toxicity is the critical effect for only one of the four chemicals, all four produce the effect, and it is conceivable that it may be a sensitive effect for the mixture. Neurological effects are not the critical effect for any of the chemicals, but three of the chemicals cause this effect at equivalent or higher exposure levels than associated with the critical effect. Thus, use of the TTD modification of the hazard index for mixtures of chemicals that do not have the same critical effect may increase the understanding of the potential impact of the mixture on public health. Additional information regarding this method is provided by Mumtaz et al. (1994a, 1997).

The development of TTDs can be analytically intensive. TTDs have been developed for a variety of chemicals in a pilot study (Mumtaz et al. 1997) and in a number of ATSDR interaction profiles (Pohl and Abadin 2008; Pohl et al. 2003, 2004, 2009; see www.atdr.cdc.gov/interaction_profiles). The derivations in the interaction profiles are subjected to a review process that is similar to that for MRLs. Currently, ATSDR Toxicological Profiles only present MRLs for subject chemicals and do not present TTDs.

3.3.4. Weight-of-Evidence (WOE) Modification to the Hazard Index Approach

As noted above, the hazard index approach 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 made by the NRC (1989) that, in recognition of the difficulties of quantifying interactions, an uncertainty factor be used to account for interactions among components of a mixture. The method was designed to modify the hazard index to account for interactions, using the WOE for interactions among pairs of mixture components. Although subsequent experience with the algorithm used to generate the interactions hazard index has revealed that it does not account for changes in proportions of mixture components in a reasonable manner, the method is useful qualitatively for predicting whether a hazard may be greater or less than indicated by the hazard index.

The method evaluates data relevant to joint action for each possible pair of chemicals in the mixture in order to make qualitative binary WOE (BINWOE) determinations for the effect of each chemical on the toxicity of every other chemical. Two BINWOEs are needed for each pair: one for the effect of chemical A on the toxicity of chemical B, and another for the effect of chemical B on the toxicity of chemical A. The BINWOE determination is a classification that indicates the expected direction of an interaction (greater than additive, less than additive, additive, or indeterminate), and scores the data qualitatively, using an alphanumeric scheme that takes into account mechanistic understanding, toxicological significance, and relevance of the exposure duration, sequence, bioassay (*in vitro* versus *in vivo*), and route of exposure. The alphanumeric terms in the classification scheme can then be converted to a single numerical score, by multiplying the corresponding direction factor by the data quality weighting factor. 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, including preparation of the ATSDR interaction profiles and a study by Mumtaz et al. (1998), has indicated that the WOE evaluations should be target-organ specific.

The qualitative BINWOE classifications are shown in the left column of Table 4 and the direction factors and data quality weighting factors are shown in the far right column. An alphanumeric (qualitative) BINWOE classification of >II.B.2.a.i for the effect of one chemical on the toxicity of another thus 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 BINWOE score is $+1(0.71)(0.71)(0.79)(1)(1)=+0.40$.

Table 4. 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. <i>In vivo</i> data	1.0
b. <i>In vitro</i> 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 WOE approach, focusing on application of the BINWOE scores to hazardous waste site assessment, was suggested by Mumtaz and Durkin (1992). This approach was recommended 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 WOE method was developed for assessing interactions for noncarcinogenic effects, the qualitative WOE method is equally applicable to assessing interactions for carcinogenic effects.

The WOE method has undergone evaluation, and appeared to perform well qualitatively (Mumtaz and Durkin 1992; Mumtaz et al. 1994a). 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]).

Possible applications of the qualitative BINWOE approach during refined Tier 3 analysis (see Section 2.4) include qualitative assessment that a hazard index is overprotective when evidence indicates that dose responses are less-than-additive or under-protective when evidence indicates that dose responses are greater-than-additive for any two (or more) components in the evaluated mixture.

A modification of the original WOE method was adopted as part of EPA's mixtures guidance (EPA 2000). This modification includes a slightly different classification scheme and a method of calculating an interactions-modified hazard index. The method encourages greater use of quantitative interaction data through the use of magnitude-of-interaction factors for each chemical pair. The classification scheme, while more integrated in nature, requires more judgment, and the type of quantitative interaction data required to estimate the magnitude factor is rarely available (see Boobis et al. 2011). The algorithm for this modification appears to handle changes in proportions of mixture components more reasonably than does the original algorithm, but additional evaluation with regard to predicting experimental results is desirable.

A basic assumption of both WOE methods is that interactive interference will not be significant. For example, if chemicals A and B interact in a certain way, the presence of chemical C will not cause the interaction to be substantially different. Thus, the assumption is that pairwise interactions will dominate in the mixture and will adequately represent all of the interactions.

Additional detail regarding both methods is provided in Appendix B.

3.3.5. Relative Potency Factor (RPF) Approaches (including Toxicity Equivalency Factor [TEF] Approaches)

RPF approaches are developed and used to evaluate mixtures of related chemicals that are assumed to be toxicologically similar, for cases in which dose-response data for one chemical in the chemical group (termed the index chemical or compound) are sufficient to derive a guidance value (e.g., an MRL, RfD, or cancer slope factor), but dose-response information for the other chemicals is less complete (EPA 2000). RPF approaches require both exposure and toxicity data and scale exposure concentrations of the non-index chemicals relative to the potency of the index chemical using scaling factors (i.e., RPFs) based on a specific toxic effect, route of exposure, or duration of exposure. A TEF approach is a special type of RPF approach for a group of chemicals sufficiently well studied to have confidence that the scaling factors (i.e., TEFs) are applicable to all health endpoints, all routes of exposure, and all durations of exposure. Compared with a generalized RPF approach, a TEF approach is based on more high-quality and abundant mechanistic data yielding considerable certainty about the MOA leading to all shared toxic effects from members in the group (EPA 2000). In essence, a TEF may be developed if there is confidence that a single MOA or toxicity pathway is shared by all members of the group. The classic example of a TEF approach is the one developed for dioxins and dioxin-related compounds, which share a key event (binding to the aryl hydrocarbon receptor) leading to downstream adverse effects (see next paragraph).

The most widely accepted TEF approach is used with the CDDs and structurally related chemical classes such as the chlorinated dibenzo-*p*-furans (CDFs) and the coplanar PCBs that are expected to have a common mechanism of action in producing common adverse outcomes (Ahlborg et al. 1994; ATSDR 1998b; EPA 1989b, 1994, 2010b 2012b; Safe 1998; Van den Berg et al. 1998, 2006). This method estimates TEFs for the various congeners in the mixture based on the key assumptions that CDD and CDF congeners produce nonneoplastic and neoplastic effects through a common receptor-mediated MOA (aryl hydrocarbon receptor), and act in a dose-additive manner. The TEF approach uses data from *in vitro* and *in vivo* studies comparing the potency of individual congeners to produce toxic or biological effects, with that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the best-studied of this chemical class. Relative potencies are calculated from these studies as the ratio of the EC₅₀ for a congener to the EC₅₀ for 2,3,7,8-TCDD (Van den Berg et al. 2006). 2,3,7,8-TCDD is assigned a TEF of one, and TEF values for the other congeners are determined by an evaluation of the range of relative potency estimates from the available studies (Van den Berg et al. 2006). The most recent consensus values for TEFs for CDDs, CDFs, and dioxin-like PCBs were determined by an expert panel convened by the WHO (Van den Berg et

al. 2006). A 2011 expert consultation evaluated the possible inclusion of brominated analogues of the dioxin-like compounds in the WHO TEF scheme, and recommended the use of similar interim TEF values for brominated and chlorinated congeners for human health risk assessment (Van den Berg et al. 2013).

To assess exposure to a specific mixture of CDDs and dioxin-like compounds, the concentrations of congeners present in a mixture in environmental media are determined and multiplied by their TEF values and then summed to give the total 2,3,7,8-TCDD toxic equivalents (TEQs) of the mixture:

$$TEQs = \sum_{i=1}^n C_i \times TEF_i \quad (4)$$

where C_i is the concentration and TEF_i is the TEF for the i^{th} component of the mixture. The TEQ thus represents the concentrations of all of the components as an equivalent concentration of the index chemical, 2,3,7,8-TCDD, based on the assumption of dose additivity. The TEQs are used in exposure models to estimate intakes for target populations and specific exposure scenarios. An index of hazard for noncancer health effects is estimated by comparing (via HQs) the TEQ intake with the appropriate MRL for 2,3,7,8-TCDD or other health-based criteria (e.g., the EPA RfD for 2,3,7,8-TCDD) (ATSDR 1998b, 2008b; De Rosa et al. 1997a, 1997b, 1997c, 1998; EPA 2010b; Mumtaz and Hertzberg 1993; Pohl et al. 1995). If the ratio of the TEQs to the guidance value for 2,3,7,8-TCDD is >1 , there is concern for increased risk of hazard; values <1 do not merit concern for increased risk. For cancer risk assessment, an estimate of cancer risk is obtained by multiplying the TEQ (in appropriate units of mg/kg/day or mg/m³) by a cancer slope factor or unit risk for 2,3,7,8-TCDD (EPA 1994, 1996; Mumtaz and Hertzberg 1993).

This TEF approach is considered suitable for the assessment of health effects of dioxin-like compounds that are mediated through the aryl hydrocarbon receptor, but is not applicable for those that are not (ATSDR 1998b; Van den Berg et al. 2006). Aryl hydrocarbon receptor mediation is thought to be a key event in the mechanism of action for effects produced by this class of chemicals including carcinogenicity, immunotoxicity, and developmental and reproductive toxicity (the basis for oral MRLs and the EPA IRIS [2012] RfD for 2,3,7,8-TCDD) (ATSDR 1998b; EPA 2010b, 2012b; Van den Berg et al. 2006). Limitations to this method are that: (1) some of the nondioxin-like PCB congeners have been shown to inhibit or enhance responses to 2,3,7,8-TCDD, depending on dose and assay system (Birnbaum

and DeVito 1995; Pohl and Holler 1995; Safe 1998); (2) some PCB congeners with many relative potency studies have a very broad range of relative potency estimates (Safe 1998; Van den Berg et al. 2006); and (3) a slope factor for 2,3,7,8-TCDD is not available on the EPA IRIS (2012). The TEF approach continues to evolve and undergo additional testing and validation. ATSDR considers the approach less suitable for PCBs, and has derived MRLs for PCBs (ATSDR 2000b). ATSDR uses the TEF method as a tool for assessing health effects of dioxin and dioxin-like compounds (primarily CDDs and CDFs) in soil (ATSDR 1998b, 2008b; De Rosa et al. 1997a, 1997b, 1997c, 1998). The most recent consensus TEF values presented by WHO (Van den Berg et al. 2006) are part of the methods used by ATSDR (2008b) and EPA (2010b) to assess human health risks of mixtures with dioxins and dioxin-like compounds. Results from *in vivo* studies of defined mixtures of dioxins and dioxin-like compounds indicated that WHO TEF values predicted mixture toxicity within a factor of ≤ 2 (Fattore et al. 2000; Gao et al. 1999; Hamm et al. 2003; Walker et al. 2005), providing evidence that the dose-additivity assumption in the TEF approach for dioxins and dioxin-like compounds is useful. Evidence that bioavailability of CDDs and CDFs can be limited in soils, and the fact that most TEFs for CDDs, CDFs, and dioxin-like PCBs are based on data from studies of laboratory animals fed test compounds in food, has led to recommendations that adjustments be made to account for decreased bioavailability in soil, when risks are assessed from exposures to dioxins and dioxin-like compounds in soil (EPA 2007a, 2010a; Van den Berg et al. 2006).

An RPF approach has been developed for nonsubstituted PAHs that have been classified as B2 carcinogens by EPA (ATSDR 1995b; EPA 1993). The RPFs (termed estimated order of potency by EPA [1993]) were estimated on the basis of potency relative to that of benzo[a]pyrene in mouse skin tumor studies. RPFs for a wider number of individual nonsubstituted PAHs (up to 24) have been developed by a number of groups (see Jarvis et al. [2014] for review). Benzo[a]pyrene is the best-studied member of this class and has a cancer OSF available on IRIS (1998a). Similar to the TEF approach, the concentrations of PAHs with RPFs are first determined in environmental media. Exposure models are then used to estimate oral intakes of each PAH for target populations, and individual PAH intakes are converted to benzo[a]pyrene equivalents with the appropriate RPF. The benzo[a]pyrene equivalents are then summed and multiplied by the benzo[a]pyrene cancer slope factor to obtain an estimate of the cancer risk in the target population from the carcinogenic PAHs in the mixture. The mechanistic underpinnings of the RPF approach for the PAHs are less compliant than CDDs with the assumption of a single common, mechanism-of-action key event, as multiple mechanisms are likely involved for different PAHs (see Boström et al. 2002; Jarvis et al. 2014). In addition, some of the same issues noted for the application of the TEF approach for CDDs also are issues for the use of the RPF approach for PAHs,

including evidence for greater-than-additive and less-than-additive interactions among binary and more complex mixtures of PAHs and the wide range in published RPF values for many individual PAHs (see Jarvis et al. [2014] for review). Several reports have indicated that the RPF approach may be inadequate for predicting carcinogenic responses in mouse-skin tumor-initiation studies of complex PAH-containing mixtures or certain PAHs (e.g., dibenzo[def,p]chrysene) having mechanisms of action different from those of benzo[a]pyrene (Courter et al. 2008; Siddens et al. 2012; Tilton et al. 2015). Other reports suggest that the mutagenic activities of extracts of PAH-contaminated soil are inadequately predicted by dose addition and RPFs due to competing factors of: (1) possible less-than-additive, metabolism-related, interactions among components and (2) contributions from non-identified mutagenic components in the extracts (Lemieux et al. 2015, 2008). However, in these studies, mutagenic activities predicted by dose addition of PAH components were mostly within 2–4-fold of observed mutagenic activities of nonpolar fractions of extracts of 10 soils contaminated with complex PAH-containing mixtures (Lemieux et al. 2015, 2008).

EPA OPP has developed cumulative risk assessments for classes of pesticides whose members produce common effects by a common mechanism using an RPF approach coupled with a POD/Margin of Exposure (MOE) approach as an index of risk (EPA 2002b). The EPA OPP approach for cumulative risk assessments 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 OPP cumulative risk assessments each began with a WOE evaluation identifying a group of chemicals that produce a common effect by a common mechanism (EPA 2002b). Using this type of evaluation, OPP determined that there was sufficient evidence for a common effect by a common mechanism for five pesticide classes (three insecticide classes and two herbicide classes) including organophosphates (EPA 2006b), N-methyl carbamates (EPA 2007b), pyrethrins/pyrethroids (EPA 2011b), triazines (EPA 2006d), and chloroacetanillides (EPA 2006c), but insufficient evidence for members of the thiocarbamate class (EPA 2001a) or members of the dithiocarbamate class (EPA 2001b).

The index chemical for the common assessment group is selected as the representative chemical in the group with the best available dose-response data for all exposure routes under consideration. When

adequate data are available, RPFs are determined by dose-response modeling of a common end point (pertinent to the common mechanism of action) to arrive at a POD (such as a BMD₁₀) and dividing the POD for each component by that of the index chemical (a unitless number). Concentrations of member components in food and environmental media are converted to index chemical equivalents by multiplying the concentrations by the appropriate RPF, and then equivalent concentrations are summed. The summed equivalent concentrations are used in exposure models to estimate total index chemical equivalent intakes for target populations for multiple pathways and exposure scenarios. The ratio between the POD for the index chemical (POD_{index chemical}) and the total index chemical equivalent intake (i.e., exposure) is termed the MOE, which is used as EPA OPP's indicator of risk:

$$\text{Margin of exposure (MOE)} = \text{POD}_{\text{index chemical}} \div \text{exposure to index chemical equivalents}$$

Uncertainties in the exposure assessment and toxicity database are considered in selecting a suitable target MOE to indicate concern for increased risk of the common effect and characterizing the risk for target populations and exposure scenarios. An illustration of the whole process can be found in the description of the EPA (2007b) cumulative risk assessment for N-methyl carbamates in Appendix C.8 of this document. Wilkinson et al. (2000) have argued that the POD/MOE approach is more transparent than the hazard index approach, because the application of data-derived uncertainty factors and default policy-driven uncertainty factors are separated in the POD/MOE approach, but masked within the RfDs or MRLs used in the hazard index approach.

3.3.6. Total Cancer Risk Approach

A response-addition approach has been recommended for the assessment of risk from mixtures of carcinogenic chemicals (De Rosa et al. 1993; EPA 1986, 2000; Mumtaz et al. 1994a; NRC 1989). The most conservative form of response addition, completely negative correlation of tolerances (i.e., individuals most sensitive to chemical A are least sensitive to chemical B and vice versa) was recommended by EPA (1986). Accordingly, the response or risk for the mixture is the sum of the risks for the components:

$$\text{Risk} = \sum_{i=1}^n \text{Risk}_i = \sum_{i=1}^n d_i B_i \quad (5)$$

where $Risk_i$ is the risk, d_i is the dose, and B_i is a potency parameter (slope factor or unit risk) for the i^{th} carcinogen. The equation is appropriate when risks for the individual chemicals are <0.01 and the sum of the individual risks is <0.1 (EPA 1989a). This equation is equivalent to dose addition if the dose-response curves for the chemicals are within the linear (low-dose) range, and have no threshold (EPA 1986, 2000). EPA (2000) recommends the response-addition model for independent action (as in Equation 18 of Appendix A) for cancer risk, noting that when component risks are small, the formula collapses to the simple addition of component risks (Equation 5 above). Use of the IRIS values for slope factor or unit risk result in plausible upper bounds to the lifetime excess cancer risk of the components. Concern has been raised that summing upper bound risks may lead to unreasonably high estimates of the mixture risk, but an analysis by Kodell and Chen (1994) suggested that the error in the simple sum of the upper bound risks is small relative to other uncertainties, and Cogliano (1997) concluded that the sum of the upper bound risks provides useful information regarding the overall risk from mixtures of carcinogens.

3.3.7. Applications of PBPK and PBPK/PD Models to Chemical Mixture Assessments

PBPK models for single chemicals are biological models that incorporate pharmacokinetic information (i.e., about absorption, distribution, metabolism, and elimination; also known as toxicokinetic information) to estimate internal doses of a chemical in the body from externally applied doses or concentrations. PBPK/PD models also incorporate information about the response of target tissues or cells to the chemical (i.e., pharmacodynamic information) (Caldwell et al. 2012; Mumtaz et al. 2012; Tan et al. 2011). PBPK models for single chemicals have been used to better inform human health dose-response assessment extrapolations from high doses to low doses, across species (e.g., from rats to humans), and across durations and routes of exposure (Caldwell et al. 2012; EPA 2006a; Mumtaz et al. 2012). Examples of toxicity guidance values that were developed using PBPK models for single chemicals include the EPA IRIS RfCs or cancer slope factors for dichloromethane (IRIS 2011a), trichloroethylene (IRIS 2011b), and vinyl chloride (IRIS 2003b), and the ATSDR MRLs for dichloromethane (methylene chloride) (ATSDR 2000a), 1,4-dioxane (ATSDR 2012b), cadmium (ATSDR 2012a), and trichloroethylene (ATSDR 2014). Single-chemical PBPK modeling is part of a process, termed quantitative *in vitro* to *in vivo* extrapolation, that is currently being investigated for use in extrapolating *in vitro* toxicity results to *in vivo* exposure scenarios via reverse dosimetry (Meek and Lipscomb 2015; Shin et al. 2015; Thomas et al. 2013; Wetmore 2015; Wetmore et al. 2012; Yoon et al. 2015).

Models for mixtures of two or more components have been developed by linking PBPK and/or PBPK/PD models for the individual components at points of potential pharmacokinetic or pharmacodynamic interaction, most commonly at hepatic metabolic inhibition (Andersen and Dennison 2004; Krishnan et al. 2002; El-Masri et al. 2004; Mumtaz et al. 2012; Tan et al. 2011). Following optimization and validation of the potential mechanisms of interaction by comparing model predictions of an internal dose metric or toxic outcome with experimental data, the mixture/interaction models have been used to investigate the dose-dependency of the magnitude of interactions and external exposure levels at which interactions (i.e., deviations from additivity) may or may not exist (Barton et al. 1995; Dobrev et al. 2001, 2002; Haddad et al. 1999a, 1999b, 2000a, 2000b; El-Masri et al. 1996, 2004; Krishnan et al. 2002; Pelekis and Krishnan 1997; Tardif et al. 1997). For example, Tardif et al. (1997) found that rat PBPK models for toluene, m-xylene, and ethylbenzene linked with competitive metabolic inhibition in the liver provided plausible agreement with results from gas uptake studies. Simulations of human models (scaled from the rat models) and results from volunteer studies showed that alveolar air concentrations and urinary metabolite concentrations, at exposure levels below permissible occupational exposure levels, were not significantly different between exposure to the individual components and exposure to the mixture. The results indicated the lack of an antagonistic metabolic interaction at these low exposure levels (Tardif et al. 1997). Similarly, El-Masri et al. (2004) demonstrated an interaction threshold for oral exposure of rats to a binary mixture of two organophosphorus insecticides that inhibit acetylcholinesterase after bioactivation by CYP enzymatic transformation: chlorpyrifos and parathion. Rat PBPK/PD models for each insecticide were developed to estimate blood concentrations of their metabolites and estimate kinetics of percent inhibition of free plasma acetylcholinesterase. A mixture model was developed that included interactions at: (1) the CYP enzymatic bioactivation step and (2) acetylcholinesterase binding sites. Model simulations with oral exposure to various dose levels of each insecticide alone and 1:1 mixtures indicated that the mixture model predicted responses that were increasingly smaller than the responses predicted by response additivity from the individual models at doses in the range of 1–10 mg/kg, thereby indicating antagonism (less-than-additive action) that increased with dose. No difference between the two methods became apparent at 0.08 mg/kg, the apparent interaction threshold for this binary mixture (El-Masri et al. 2004). Rat PBPK models for more complex mixtures of up to five volatile organic components (benzene, toluene, ethylbenzene, and xylenes [BTEX] and dichloromethane) have been developed that consist of PBPK models of the individual components linked by competitive metabolism in the liver (Haddad et al. 1999a, 1999b, 2000a, 2000b; Krishnan et al. 2002). The interaction-based mixture models adequately simulated measured internal dose metrics for each component following short-term inhalation exposure to various combinations and concentrations of the components. Simulations with a dichloromethane/BTEX model for humans (scaled from the rat model) indicated that competitive

metabolic inhibition would: (1) decrease hazard indices for anoxia (i.e., carboxyhemoglobin formation) from dichloromethane depending on mixing ratios with, and concentrations of, other components; (2) increase blood concentration x time profiles for each component at higher concentrations compared with dose additivity expectations with implications for prolonging acute central nervous system effects from all components; and (3) likely, at high concentrations, increase cancer risk from dichloromethane by shifting metabolism to a putatively cancer-related pathway (GSH conjugation vs. CYP) and decrease cancer risk from benzene by inhibiting formation of putatively carcinogenic reactive metabolites by CYP enzymes (Haddad et al. 2001). A noted limitation of applying this model to subchronic or chronic exposure scenarios is that it was developed with acute duration kinetics data and other points of metabolic interaction (e.g., enzyme induction) could arise with repeated exposure to the mixture (Krishnan et al. 2002).

An approach to dealing with very complex mixtures is to model fractions of the mixture as single components or lumps. This approach has been used to predict whether the metabolism of benzene to genotoxic metabolites is affected by the other components of gasoline in the mouse (Bond et al. 1998). A similar approach was proposed and partially developed for studying the acute toxicology of JP-5, a Navy jet fuel that contains a complex mixture of petroleum hydrocarbons in the C9–C18 range (Verhaar et al. 1997; Yang et al. 1998). The lumping concept for very complex mixtures has been applied to develop rat PBPK models for gasoline (Dennison et al. 2003, 2004). Results from gas-uptake studies of rats exposed for 6 hours to whole gasoline vapors or fractions of whole gasoline vapors were used to develop PBPK models that linked individual PBPK models for five individual components (n-hexane, benzene, toluene, ethylbenzene, and o-xylene) and a lumped component of the remaining evaporative components (principally hydrocarbons representing about 90% by weight of the complex mixture) by competitive metabolism in the liver. Stepwise optimization was used to estimate model parameters by comparison of time profiles of GC-measured chamber concentrations of the five individual components and the lumped component with model predictions. The internal dose metric of interest in these models was blood concentration of each individual component. Simulations with the developed rat models indicated that competitive metabolic inhibition for most of the components occurred at ≥ 300 ppm for whole gasoline vapors and ≥ 200 ppm for fractions of whole gasoline vapor that were expected to be more relevant to environmental exposure scenarios experienced by humans than whole gasoline vapors (Dennison et al. 2003, 2004).

To date, PBPK/PD models developed for mixtures have not been routinely applied in the development of risk-based guidance values for mixtures or cumulative risk assessments for specific mixtures. However,

ATSDR (2004b) used simulations from the BTEX PBPK model (Haddad et al. 1999a, 1999b, 2000, 2001; Krishnan et al. 2002) in support of recommendations for conducting exposure-based assessments of neurological, hematological, and cancer hazards from BTEX mixtures. Recommendations included a component-based hazard index approach that assumes dose additivity and uses ATSDR MRLs based on neurological impairment for neurological hazards, based on the implications from the PBPK model that joint neurotoxic action is dose additive at concentrations below about 20 ppm for each component. It was also recommended that the inhalation cancer slope factor for benzene be used for assessing cancer risk from BTEX exposures. ATSDR noted that the BTEX PBPK model simulations indicated that as exposure concentrations increase beyond 20 ppm for each component, the potential for neurotoxicity may increase and the potential for hematotoxicity/carcinogenicity may decrease beyond dose-additivity expectations due to competitive metabolic interactions among mixture components (ATSDR 2004b).

3.3.8. Approaches to Assessing Health Risks from Combined Exposure to Multiple Chemicals and Nonchemical Stressors

U.S. governmental agencies have responded to calls for developing guidance and guidelines for cumulative risk assessment for multiple chemical and nonchemical stressors (see Sexton [2012] for a historical review). Nonchemical stressors include biological (e.g., infectious microorganisms), physical (e.g., noise, vibrations), and psychosocial stressors (e.g., low socioeconomic status, dilapidated housing, residential crowding, and lack of access to health care). EPA (2003) prepared a *Framework for Cumulative Risk Assessment* that described a simple, flexible structure for conducting cumulative risk assessments, meaning “an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors.” The framework described three main phases to cumulative risk assessments (i.e., planning, scoping, and problem formulation; analysis; and risk characterization), but did not describe specific protocols or guidance for any of these phases. The EPA framework document, however, acknowledged a shift in its risk assessment processes to: (1) focus on identifying at-risk communities in contrast to the traditional focus of quantitatively estimating hypothetical individual risks for maximally exposed individuals from point sources or other types of environmental exposures to single or multiple chemicals; (2) use of qualitative or semi-quantitative data, such as broad exposure or toxicity indicators, in cases where the complexity of exposure and data deficiencies may hinder quantitative approaches; and (3) incorporate nonchemical stressors. The EPA viewed the framework as a first step in the long-term development of such guidance, and noted that incorporating “nonconventional stressors or risk factors (e.g., lifestyle, access to health care)” would need continued research. A report from a committee of the NRC (2009) reinforced the need for additional research to aid the development of cumulative risk assessment methods for multiple chemical and

nonchemical stressors, noting that EPA had not included, at that time, nonchemical stressors in quantitative or qualitative cumulative risk assessments.

The EPA (2003) discussion of incorporating nonchemical stressors into the analysis phase of cumulative risk assessments focused on a four-component concept of vulnerability of individuals or subgroups of the general population: (1) susceptibility or sensitivity related to biological differences associated with life-stage, genetics, or disease state; (2) differential exposure (i.e., disproportionate exposure relative to other groups or individuals) extending to historical exposure; (3) differential preparedness to withstand insult from a stressor; and (4) differential ability to recover from insults from a stressor (i.e., resiliency). Lack of access to health care, income differences, unemployment, or lack of insurance were given as examples of social factors that may influence a community's ability to prepare or recover from an insult from a stressor. Within this concept of vulnerability, Lewis et al. (2011) prepared a list of potential indicators of individual or community vulnerability compiled from several sources (Cal/EPA 2010; deFur et al. 2007; Morello-Frosch and Shenassa 2006; O'Neill et al. 2003). The list included indicators of biological susceptibility and sensitivity, such as inherited diseases, genetic polymorphisms, age, developmental or physiological stage (e.g., pregnancy), race/ethnicity/culture, mental health-related coping skills, and low intelligence or birth weight. Indicators of differential exposure related to either individual or community vulnerability included old/substandard housing, substandard sanitation, increased air pollutant exposure, and proximity to industrial release sites or hazardous waste sites. Indicators of either differential individual or community preparedness and recovery included low socioeconomic status, family instability, inadequate nutrition or food supply, limited health care access or insurance, high incidence of obesity, smoking or drug addiction, crime and violence, and lack of general community resources. Similarly, ATSDR (2014) developed a social vulnerability index approach that enables public health officials and emergency planners to identify and map communities that are socially vulnerable http://grasp.cdc.gov/grasp_intranet/grasp_svi.aspx. Several efforts to develop tools to incorporate nonchemical stressors such as those associated with differential exposure and differential preparedness and ability to recover have been reported, but the tools are qualitative in nature and their usefulness is limited to ranking or prioritizing communities for further cumulative risk assessment investigations (Alexeeff et al. 2012; NJDEP 2009; Su et al. 2009). For example, The California Environmental Protection Agency is developing a screening tool for assessing differential cumulative impacts in different geographical regions incorporating chemical pollution data and community public health characteristics (Alexeeff et al. 2012). In a pilot analysis and application of the method to 30 ZIP mail code regions in California, ranking scores for exposure indicators (range of 1–10 based on PM_{2.5} and ozone air concentrations, EPA Toxics Release Inventory data, traffic volumes, and pesticide use), public health

effects (range of 1–5, based on data for birth weight, heart disease and cancer mortality, and asthma hospitalization), and environmental effects (range of 1–5, based on numbers of hazardous waste and clean-up sites and spills and leaks from underground fuel tanks) were added together and then multiplied by the sum of scores for sensitive populations (score of 1–3, based on census data for percent <5 and >65 years of age) and socioeconomic factors (score of 1–3, based on percent with less than a high school education, median household income, and percent below 2 times the national poverty level). Cumulative impact scores could range between 6 and 120. Alexeeff et al. (2012) emphasized that the method does not quantitatively estimate human health risks, but is a screening-level ranking tool. In another approach, Su et al. (2009) used air contaminant concentration data for three pollutants (NO₂, PM_{2.5}, diesel particulate matter) to estimate environmental hazards and percentage of residents who are non-white or percentages of residents with incomes lower than 2 times the national poverty level to measure socioeconomic characteristics of census-based tracts (i.e., regions) within Los Angeles county and related the cumulative environmental hazards (combined either with a population-weighted multiplicative model or an additive model) to either of the socioeconomic characteristics with a ranking index (termed a cumulative hazard inequality index) to explore demographic inequalities in environmental hazard. The calculated indices reinforced the concept that demographic inequalities existed using either socioeconomic characteristic. Lewis et al. (2011) noted that these ranking approaches do not quantitatively attribute relative contributions of chemical and nonchemical stressors to health risks, and that additional research is needed to develop such quantitative approaches.

Physical stressors known to affect similar target organs as chemicals are likely to be incorporated into quantitative health impact or risk assessments with multiple chemical stressors sooner than psychosocial stressors, because methods to measure the intensity of exposure to physical stressors are available and characterization of dose-response relationships is thus more straightforward (Rider et al. 2014). Physical stressors with evidence that they can produce toxicity or modify the toxicity of chemicals include sunlight, noise, radiation, and temperature (Rider et al. 2014), but quantitative cumulative risk assessments including these stressors with chemical stressors that may affect similar toxicity targets are rare.

A recent case study shows how these assessments may proceed. Evans et al. (2014) conducted a screening-level cumulative risk assessment for potential hearing impairment from joint exposure to traffic-related noise and airborne concentrations of three volatile organic compounds (VOCs) (toluene, ethylbenzene, and mixed isomer xylenes) in San Francisco County, California. A component-based hazard index approach was used based on a dose-additivity assumption for these four components

determined to cause hearing impairment in other epidemiology and/or toxicology studies. Acceptable or “safe” levels for chronic exposure to each of the components were used to calculate HQs for each component: 70 decibels (dB) for noise as determined by WHO (1999) and EPA IRIS RfCs of 5 mg/m³ for toluene (IRIS 2007), 1 mg/m³ for ethylbenzene (IRIS 1998b), and 0.1 mg/m³ for xylenes (IRIS 2003c). A noise map for San Francisco County was developed using a model of traffic-induced noise levels developed by the U.S. Federal Highway Administration. Geographical block groups within the county as determined by the 2000 U.S. census were the geographical units for the assessment. To estimate noise levels within each block group, the modeled noise level of streets within each block were averaged and placed into one of four noise categories: 45–60, 61–65, 66–70, and 71–75 dB. Because appropriate block-level data were not available for airborne concentrations of the VOCs, these were estimated (extrapolated) by quantile regression modeling of sociodemographic data (race, gender, education, and smoking status: these types of data were also available for the San Francisco block groups) and personal air VOC concentration data from 648 individuals with both types of data in the 1999–2000 U.S. National Health and Nutrition Examination Survey (NHANES). Hazard indices for each block were calculated by adding the block-level HQs for noise, toluene, ethylbenzene, and mixed xylenes. County-averaged hazard indices ranged from 0.8 for the tenth percentile of combined VOC exposure and the low noise category (45–60 dB) to 1.7 for the 90th percentile of combined VOC exposure and the high noise category (71–75 dB).