1. INTRODUCTION

1.1. OVERVIEW

Assessing the health impact of chemicals or other stressors (e.g., noise, radiation) in the environment is complicated by the reality that most toxicological testing is performed on single chemicals or physical agents, but human exposures are rarely limited to single agents. Exposures resulting from hazardous waste sites or other releases into the environment generally involve more than one toxic substance agent and other stressors (ATSDR 2005a; Carpenter et al. 2002; De Rosa et al. 1996; Hansen et al. 1998; Johnson and De Rosa 1995; MacDonell et al. 2013; Mumtaz et al. 2007, 2011). This occurrence leads to concerns that exposures to multiple chemicals or other stressors may impact public health in ways not anticipated by assessing the impacts of each agent alone.

This manual presents a recommended approach to assess potential health impacts of exposures to multiple chemicals in ATSDR public health assessments and health consultations. It is an update of, and replaces, the 2004 ATSDR *Guidance Manual for the Joint Toxic Action of Chemical Mixtures* (ATSDR 2004a). This document also provides overviews of the scientific principles and evidence guiding the recommended approach.

ATSDR's public health assessments and U.S. Environmental Protection Agency (EPA) quantitative risk assessments both address potential human health effects of environmental exposures to chemicals and other agents, but they are approached differently and used for different purposes (ATSDR 2005a). ATSDR's public health assessments consider past, current, and future exposures to chemicals of concern, evaluate toxicological or epidemiological data for chemicals or mixtures of concern, and compare epidemiological or toxicological dose-response data or public health guidance values (Minimal Risk Levels [MRLs] and EPA cancer slope factors) with population exposure estimates to arrive at indices of human health impacts. The ATSDR public health assessment process focuses closely on site-specific exposure conditions and health outcome data, and considers specific community health concerns to arrive at qualitative recommendations to reduce or prevent harmful exposures or take other public health actions (ATSDR 2005a). ATSDR (2005a) guidelines for public health assessments call for early and continued coordination and communications with community members and representatives, principal responsible parties (i.e., stakeholders), EPA, and other federal, state, and local agencies. Effective coordination and communication with all interested parties throughout the process can lead to harmonization and acceptance of recommended cleanup goals (from EPA) and public health actions. In contrast, EPA's quantitative risk assessments are used as part of investigations to determine the extent to which site

remedial action (e.g., clean up) or restricted use actions (e.g., for pesticides) are needed. EPA risk assessments consider current and potential future exposures for chemicals of concern, evaluate toxicological or epidemiological data for chemicals or mixtures of concern, and compare public health guidance values (e.g., reference doses [RfDs], reference concentrations [RfCs], and cancer slope factors) for mixtures, if available, or components of mixtures, with exposure estimates for specific populations to arrive at numerical estimates of health risk if no cleanup occurs.

This first chapter of this framework manual discusses concepts and definitions that are used in assessments of potential health impacts or risks from exposure to multiple chemicals and other stressors. Chapter 2 presents ATSDR's recommended 3-tiered approach, and Chapter 3 discusses options and issues related to assessing public health impacts from exposure to multiple chemicals and nonchemical stressors. Appendices A and B provide additional background information not covered in Chapter 3, and Appendix C describes approaches recommended by other U.S. agencies and other national and international agencies.

1.2. SOME CONCEPTS AND DEFINITIONS

With the evolvement of methods to assess health impacts or risks from exposure to multiple chemicals or other stressors, various terminologies have developed that warrant some explanation in this framework (see Tables 1 and 2 for definitions of selected chemical mixture and chemical interaction terms).

"Exposure to a chemical mixture" has traditionally been used to refer to combined environmental exposure to multiple chemicals. Such mixtures can be simple, being comprised of a relatively small number of known "components", or complex, being comprised of many chemicals, often of different chemical classes. Simple mixtures may be associated with hazardous waste sites, when the number of components of concern identified in environmental media (i.e., those components presenting exposures near to or above public health guidance values) are small. The composition of complex mixtures may not be fully characterized and can vary dependent on production conditions and time since release in the environment.

Table 1. Definitions of Chemical Mixture Terms

Mixture	Any set of multiple chemicals, regardless of source and spatial or temporal proximity that may jointly contribute to actual or potential health effects in a population.
Components	The chemicals that make up a mixture.
Simple mixture	A combination of a relatively small number of chemicals that has been identified and quantified (e.g., the components of concern for a community near a hazardous waste site may constitute a simple mixture).
Complex mixture	A complex mixture has very many chemicals, often of different chemical classes. The composition of complex mixtures may not be fully characterized and can vary dependent on production conditions and time since release in the environment. Components of complex mixtures may be generated simultaneously from a single source or process (e.g., tobacco smoke), intentionally produced as a commercial product (e.g., gasoline, jet fuels, mixtures of pesticides), or coexist in environmental media as a consequence of waste disposal operations or release of components into the environment from multiple sources.
Original mixture	Any combination of all chemicals that are released into the environment at a specific point in time and location. The composition of the original mixture can change with time and location due to differential fate and transport properties of the components.
Mixture of concern	The actual mixture being evaluated in a site-specific risk assessment; often referred to as the “whole” mixture.
Sufficiently similar mixture	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, similar mixtures and their components have similar environmental fate and transport properties, and produce similar health effects, whereas dissimilar mixtures do not.
Chemical class	A group of chemicals that are similar in chemical structure and in eliciting similar biochemical sequences of events related to toxic effects, and which frequently occur together in the environment, usually because they are generated by the same process, such as manufacturing or combustion (e.g., PCBs, CDDs, PAHs).
Components of concern	The chemicals in a mixture that are likely contributors to health hazard either because their individual exposure levels approach or exceed health guidelines, or because joint toxic action with other components, including additivity or interactions, may pose a health hazard.
Index chemical	The chemical selected as the basis for standardization of toxicity of components in a group of chemicals or agents (e.g., TCDD for the assessment of dioxin-like compounds; benzo[a]pyrene for the assessment of carcinogenic PAHs).
Indicator chemical(s)	A chemical (or chemicals) selected to represent the toxicity of a mixture because it is characteristic of other components in the mixture and has adequate dose-response data (e.g., benzene has been suggested as an indicator chemical for a specific fraction of gasoline).
Aggregate exposure	The combined exposure of a population to a specific agent or stressor via multiple relevant routes, pathways, and sources.
Aggregate risk	The risk resulting from aggregate exposure to a single agent or stressor.
Cumulative risk	Cumulative risk is the combined risks from aggregate exposures to multiple agents or stressors. Cumulative risk assessment is an analysis, characterization, and possible quantification of the combined risks to health from multiple agents or stressors.

Sources: EPA 1986, 1988, 2000, 2003; Fay and Feron 1996; Hertzberg et al. 1999

CDD = chlorinated dibenzo-*p*-dioxin; PAH = polycyclic aromatic hydrocarbon; PCB = polychlorinated biphenyl; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Table 2. Interactions/Mixtures Terminology[a,b]

Interaction	When the effect of a mixture is different from the expectation of additivity based on the dose-response relationships of the individual components. In this context, additivity as "no-interaction" is the null hypothesis.
Additivity	When the effect of the mixture can be estimated from the sum of the exposure levels (weighted for potency in dose or concentration additivity) or the probabilities of effect (response additivity) of the individual components. In dose additivity (also called concentration additivity), each chemical behaves as a dilution of every other chemical in the mixture. Most stringently, each chemical contributes to the production of a common adverse outcome via a common mechanism of action. Less stringently (for screening level assessments), each chemical contributes to the production of a common adverse outcome regardless of mechanism of action. In response additivity (also called independent action), components of a mixture act independently of each other and probabilities of response to components are added.
No apparent influence	When a component that is not toxic to a particular biological system does not influence the toxicity of a second component on that system.
Synergism	When the effect of a mixture is greater than that estimated by additivity. Synergism is defined in the context of the definition of no interaction, which is usually dose additivity or response additivity. The use of "greater-than-additive" is preferred over the use of the term synergism.
Potentiation	When a component that is not toxic to a particular biological system increases the effect of a second chemical on that system.
Antagonism	When the effect of a mixture is less than that estimated by additivity. Antagonism is defined in the context of the definition of no interaction, which is usually dose additivity or response additivity. The use of "less-than-additive" is preferred over the use of the term antagonism.
Inhibition	When a component that does not have a toxic effect on a particular biological system decreases the apparent effect of a second chemical on that organ system.
Masking	When the components produce opposite or functionally competing effects on the same biological system, and diminish the effects of each other, or one overrides the effect of the other.

[a]Where effect is incidence or measured response, and additivity commonly is dose or response additivity.
[b]Based on definitions in EPA (1988, 2000, 2003), Hertzberg et al. (1999), Hertzberg and MacDonell (2002), and Mumtaz and Hertzberg (1993).

Components of complex mixtures may be generated simultaneously from a single source or process (e.g., tobacco smoke, coke oven emissions, diesel engine emissions), intentionally produced as a commercial product (e.g., gasoline, jet fuels, transformer coolants containing mixtures of polychlorinated biphenyls [PCBs]), or coexist in environmental media as a consequence of waste disposal operations or release of components into the environment from multiple sources.

Other terms related to aggregate and cumulative risk assessment have increased in frequency of use within the past 10–15 years (EPA 2003; see Table 1). This manual presents ATSDR framework for assessing health impacts from combined exposure to multiple chemicals and nonchemical stressors; the ATSDR assessment process fits within the category of cumulative risk assessment.

Assessments of health impacts or risks of simple or complex mixtures can be based on exposure data and epidemiologic or toxicologic data for the original mixture. However, following release to the environment, *simple or complex mixtures can change with time and distance from the original release site, due to the differential fate and transport of their components.* For example, immediately following a release of gasoline to soil, inhalation exposure to the more volatile components, especially the low molecular weight alkanes, may be a concern. Contamination of groundwater and surface water with the more soluble components (such as benzene, ethylbenzene, toluene, and xylene) may occur over a period of weeks to years, possibly impacting drinking water. The less mobile constituents, such as aliphatic or aromatic hydrocarbons with ≥16 carbons, may tend to remain in the soil at the site of the original release for extended periods. Thus, people living near the site of release to the environment are likely to be exposed to subsets of the original chemicals at different proportions than in the complete original mixture, and chemical composition may continue to change over time. Health guidance values based on toxicological or epidemiological data for the original mixture released into the environment may not be applicable to the actual exposures experienced by people living in the vicinity of the release.

One concern for ATSDR in terms of public health is that joint toxic action or interactions among components of a mixture of concern may increase the health hazard impact above what would be expected from an assessment of each component singly. A particular issue is whether a mixture of components, each of which is present at less than guidance concentrations, may be hazardous due to additivity, interactions, or both.

As mentioned above, toxicological interactions can either increase or decrease the apparent toxicity of a mixture relative to that expected on the basis of dose-response relationships for the components of the

mixture. Table 2 provides definitions of terms used in describing the results of interactions studies. These are the definitions that will be used in this document; other definitions exist. Some of the terms, such as dose additivity or response additivity, refer to the lack of interactions. Interactions are defined as deviations from the results expected on the basis of additivity, either dose additivity or response additivity ("no-interactions"-based hypotheses). Ultimately, the various types of interaction and noninteraction can be sorted into three categories: greater-than-additive (synergism, potentiation), additive (additivity, no apparent influence), and less-than-additive (antagonism, inhibition, masking).

The early toxicology literature contains many claims of synergism or antagonism based on study designs that were inadequate to support the claims (Boobis et al. 2011; Borgert et al. 2001; Krishnan and Brodeur 1991). A typical inadequate design might involve exposure to component A and component B at subthreshold exposure levels, and when some biological response to the mixture was observed, a claim of synergism might have been made. However, depending on the individual dose-response relationships for the components, the observed response could be consistent with dose addition (a "no-interactions" hypothesis), greater-than-additive, or less-than-additive joint toxic action (see Appendix A.3.3 for more discussion of evaluating interaction studies). Borgert et al. (2001) presented five criteria that are useful for evaluating toxicological interaction studies and designing valid toxicological tests of interactions:

1. Dose-response curves for the mixture components should be adequately characterized.
2. An appropriate "no-interaction" hypothesis should be explicitly stated and used as the basis for assessing synergy and antagonism.
3. Combination of mixture components should be assessed across a sufficient range (of exposure levels and mixing ratios) to support the goal of the study.
4. Formal statistical tests should be used to distinguish whether the response produced by a dose combination is different (larger or smaller) from that predicted by the 'no-interactions" hypothesis (dose addition or response addition).
5. Interactions should be assessed at relevant levels of biological organizations.

The major mechanisms for toxicant interactions are direct chemical-chemical, pharmacokinetic, and pharmacodynamic mechanisms (Mumtaz and Hertzberg, 1993). Most of these mechanisms affect the internal concentrations of the toxicants or their active forms. Knowledge of these mechanisms for two-chemical (binary) mixtures and for classes of chemicals can be incorporated qualitatively or quantitatively into assessments of mixtures of chemicals using methods described in Chapter 3 of this document.