1. OVERVIEW

The health assessment of hazardous substances is complicated by the reality that most toxicological testing is performed on single chemicals, but human exposures are rarely limited to single chemicals. Exposures resulting from hazardous waste sites generally involve more than one hazardous substance, which may include radiation and radionuclides (ATSDR 1992; Carpenter et al. 2002; De Rosa et al. 1996; Hansen et al. 1998; Johnson and De Rosa 1995). In addition, people voluntarily expose themselves to a variety of pharmacologically active chemicals such as those in recreational drugs (alcohol and tobacco), medicines, and foods, and are involuntarily exposed to other chemicals, such as those in vehicle exhaust, drinking water, and in the workplace. 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.

The focus of this guidance is the exposure-based assessment of joint toxic action of chemical mixtures associated with hazardous waste sites, but suggestions for the appropriate consideration of non-site-related exposures also are provided. This guidance represents only the current state of knowledge in this area, and it will be revised in the future as the state of knowledge of joint toxic action of chemical mixtures develops.

1.1. INTRODUCTION

The term chemical mixture is used as "shorthand" for the concept of multiple chemical exposure. Some chemical mixtures are *intentional*—they are manufactured products, such as pesticide formulations, gasoline, or laundry detergent. Other chemical mixtures are *generated*—they are byproducts of such processes as smelting, drinking water disinfection, fuel combustion, and cigarette smoking. The chemical mixtures of concern at hazardous waste sites often are *coincidental*—they consist of unrelated chemicals from different sources, deposited separately at the site, but having the potential to reach the same "receptor population" by their presence in or migration into the same medium (commonly groundwater), or through a combination of media and pathways. (A receptor population is a population that is exposed or potentially exposed through identified exposure routes to contaminants at an exposure point [ATSDR 1992]). These categories of mixtures describe how the mixture originated.

ATSDR and other agencies such as the Environmental Protection Agency (EPA), National Institute of Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA), and American Conference of Governmental Industrial Hygienists (ACGIH) derive health criteria, guidelines,

or regulations primarily for single chemicals and, occasionally, for intentional or generated mixtures. The health values for the mixtures generally are based on data for the mixture itself, studied as if it were a single chemical. These mixtures include polychlorinated biphenyls (PCBs), certain fuels and pesticides, coal tar volatiles, and coke oven emissions.

For mixtures that are made up of relatively heterogenous components, however, health guidelines or regulations based on data for the original mixture may not be particularly useful for some exposure scenarios. 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 ground and surface water with the more soluble components, including the BTEXs (benzene, toluene, ethylbenzene, and xylenes) may occur over a period of weeks to years, possibly impacting drinking water. The less mobile constituents such as benzo[a]pyrene may tend to remain in the soil at the site of the original release for extended periods. Thus, receptor populations are likely to be exposed to subsets of the original chemicals, and to different proportions of these chemicals than in the complete mixture. Health criteria or regulations based on toxicological data for the original mixture may not be applicable to the actual exposures resulting from a release, because *mixtures change with time and distance from the original release site, due to the differential fate and transport of their components.*

1.2. SOME CONCEPTS AND DEFINITIONS

Another set of mixture categories is useful in assessing the joint toxic action of chemical mixtures; these categories include simple and complex mixtures. Mixture definitions used in assessing the consequences to human health of joint toxic action of chemical mixtures are provided in Table 1.

In the absence of data and health criteria for the mixture of concern or a sufficiently similar mixture, the approach recommended by ACGIH (2000), EPA (1986, 1989a, 1990, 2000), NIOSH (1976), and OSHA (1993, 2001) has been to use the exposure and health criteria for the individual components of the mixture. The process involves evaluation of whether the exposures or risks for the components can reasonably be considered as additive based on the nature of the health effects. In addition, EPA recommends an evaluation of whether toxicological interactions among the components are likely to result in greater (or lesser) hazard or risk than would be expected on the basis of additivity alone.

The concern for ATSDR in terms of public health is similar; toxicological interactions may increase the health hazard above what would be expected from an assessment of each component singly, or all

components additively. 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

Table 1. Definitions of Chemical Mixture Terms*

Mixture	Any combination of two or more chemicals, regardless of source and spatial or temporal proximity, that may jointly contribute to actual or potential effects in a receptor population.
Simple Mixture	A combination of a relatively small number of chemicals (no more than 10) that have been identified and quantified (e.g., the components of concern for a receptor population near a hazardous waste site may constitute a simple mixture).
Complex Mixture	A combination of so many chemicals that the composition of the mixture is not fully characterized, either qualitatively or quantitatively, and may be variable (e.g., cigarette smoke, diesel exhaust, gasoline).
Similar Mixtures	Mixtures having the same chemicals but in slightly different proportions or having most but not all chemicals in common and in highly similar proportions. Similar mixtures are expected to have similar fate, transport, and health effects (e.g., the jet fuel JP-5 from different sources).
Chemical Class	A group of chemicals that are similar in chemical structure and biological activity, 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, chlorinated dibenzo-*p*-dioxins [CDDs]).
Components	The chemicals that make up a mixture.
Components of Concern	The chemicals in a mixture that are likely contributors to health hazard either because their individual exposure levels 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 chemical class (e.g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD] for the assessment of dioxin-like compounds; benzo[a]pyrene for the assessment of carcinogenic polycyclic aromatic hydrocarbons [PAHs]).
Indicator Chemical(s)	A chemical (or chemicals) selected to represent the toxicity of a mixture because it is characteristic, potent, and has adequate dose-response data (e.g., benzene has been suggested as an indicator chemical for gasoline).

*Modified from EPA 1986, 1990, 2000; Fay and Feron 1996; Hertzberg et al. 1999.

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 additivity, refer to the lack of interactions. Interactions are defined as deviations from the results expected on the basis of additivity. 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).

Table 2. Interactions Terminology[a,b]

Interaction	When the effect of a mixture is different from additivity based on the dose-response relationships of the individual components.
Additivity	When the effect of the mixture can be estimated from the sum of the exposure levels (weighted for potency) or the effects of the individual components.
No apparent influence	When a component which is not toxic to a particular organ system does not influence the toxicity of a second component on that organ system.
Synergism	When the effect of the mixture is greater than that estimated for additivity on the basis of the toxicities of the components.
Potentiation	When a component that does not have a toxic effect on an organ system increases the effect of a second chemical on that organ system.
Antagonism	When the effect of the mixture is less than that estimated for additivity on the basis of the toxicities of the components.
Inhibition	When a component that does not have a toxic effect on a certain organ 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 organ 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 (1990,2000), Hertzberg et al. (1999), and Mumtaz and Hertzberg (1993).

The major mechanisms for toxicant interactions are direct chemical-chemical, pharmacokinetic, and pharmacodynamic mechanisms. Knowledge of these mechanisms for two-chemical (binary) mixtures and for classes of chemicals can support the prediction of interactions for new combinations of chemicals. Most of these mechanisms affect the internal concentrations of the toxicants or their active forms. Table 3 lists examples of these types of interactions, primarily for compounds of occupational and environmental concern. A more detailed discussion of mechanisms of interaction is provided in a related Agency document, the *Guidance for the Preparation of an Interaction Profile* (ATSDR 2001).

Table 3. Mechanistic Bases of Toxicological Interactions among Chemicals*

Basis of interaction	Examples	
	Synergism or potentiation	Antagonism or inhibition
Chemical-chemical	Formation of nitrosamines (which are carcinogenic) from noncarcinogenic nitrites and amines in the stomach (Klaassen 1996)	Ammonia, administered orally, acts as antidote by reacting with ingested formaldehyde to form hexamethylene-tetramine (Goldstein et al. 1974)
Pharmacokinetic		
Absorption	Neurotoxicity of EPN (*o*-ethyl-*o*-4-nitrophenyl phenylphosphonothioate) enhanced by aliphatic hydrocarbons due in part to increased dermal absorption (Abou-Donia et al. 1985)	Dietary zinc inhibits some aspects of lead toxicity in part by decreasing dietary lead absorption (Cerklewski and Forbes 1976)
Distribution	Increased neurotoxicity from increased lead levels in brain after treatment with disulfiram, due to formation of complex that readily distributes lead to brain (Oskarsson and Lind 1985; Oskarsson et al. 1986a, 1986b)	Selenium protects against cadmium toxicity by decreasing the concentration of cadmium in liver and kidney and by redistributing cadmium in the testes from the low to high molecular weight Cd-binding proteins (Chen et al. 1975)
Excretion	Decreased renal excretion of penicillin when co-administered with probenecid, potentiating its therapeutic effect (Levine 1973)	Arsenic antagonizes the effects of selenium in part by enhancing the biliary excretion of selenium (Levander and Argrett 1969)
Metabolism	Organophosphorous compounds (profenfos, sulprofos, DEF) potentiate the toxicity of fenvalerate and malathion by inhibiting esterase which detoxifies many pyrethroid insecticides and also malathion (Gaughan et al. 1980)	Selenium inhibits 2-acetylamino-fluorene-induced hepatic damage and tumorigenesis in part by shifting metabolism towards detoxification (ring hydroxylation) relative to metabolic activation (9N-hydroxyla-tion) (Marshall et al. 1979)
Pharmacodynamic		
Interaction at same receptor site (receptor antagonism) or target molecule	No examples expected	Atropine antagonizes organophosphate poisoning by blocking acetylcholine receptor sites (Goldstein et al. 1974; Klaassen 1996)
Interaction at different sites on same molecule	Tiazofurin and selenazofurin metabolites bind to different sites on inosine monophosphate dehydro-genase to synergistically inhibit its activity (Chou and Rideout 1991).	Antagonism of copper binding to DNA by other divalent cations (Sagripanti et al. 1991)
Interaction among different receptor sites or targets	Potentiation of hepatoxicity of carbon tetrachloride by chlordecone inhibition of hepatocellular repair (Mehendale 1994)	Opposing effects of histamine and norepinephrine on vasodilation and blood pressure (functional antagonism) (Levine 1973)

*Adapted from EPA (1990) and Mumtaz and Hertzberg (1993).

The literature on interactions is limited in its direct applicability to mixtures associated with hazardous waste sites. As of 1991, the majority of interactions studies on chemicals were in the form of studies of the acute lethality or hepatotoxicity of binary mixtures administered by gavage or intraperitoneal injection to experimental animals (Hertzberg and Durkin 1994; Mumtaz and Durkin 1992; Mumtaz and Hertzberg 1993). Many of these studies employed a sequential treatment protocol, in which a chemical that alters metabolism or physiology in a known manner was administered before the chemical of concern, in order to investigate the impact on the second chemical's toxicity. This study design provided data useful in elucidating the mechanism of action of the second chemical, but not so useful in understanding potential interactions involving low level, long-term simultaneous exposure to chemicals in drinking water, food, soil, and air. Because of these and other limitations, a weight-of-evidence approach to the assessment of interactions may be useful.

Recently, another option for assessing interactions has become available: PBPK/PD modeling of mixtures. Although such models are available for very few mixtures at present, this is an area of active research and is promising because it supports the exploration of a variety of exposure scenarios.