NCEA-C-0148
April 1999
External Scientific Peer Review Draft

Guidance for Conducting Health Risk Assessment of Chemical Mixtures

Risk Assessment Forum Technical Panel

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NOTICE

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PREFACE

The U.S. EPA's Risk Assessment Forum published the *Guidelines for the Health Risk Assessment of Chemical Mixtures* in 1986 (U.S. EPA, 1986). The Environmental Criteria and Assessment Office (now the National Center for Environmental Assessment) followed this with the production of a *Technical Support Document on Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1990). The 1986 Guidelines represent the Agency's science policy and are a procedural guide for evaluating data on the health effects from exposures to chemical mixtures. The Guidelines promote the standardization of risk methods across U.S. EPA Programs and regional offices. They are not intended to be a regulatory document and are not the result of any specific legislation. The Guidelines address the hazard identification, dose-response and risk characterization parts of the risk assessment paradigm as they apply to chemical mixtures. The Guidelines further direct the risk assessor to fully describe uncertainties, assumptions, limitations, and the scientific basis and rationale for decisions considered in a risk assessment. The Guidelines represent the primary Agency methodology for assessing the risk from exposure to multiple chemicals and where possible incorporate state-of-the-art research methodology for this purpose. Because the science of environmental risk assessment continues to evolve, the U.S. EPA's Risk Assessment Forum established a Technical Work Panel to ensure that the advances in the area of chemical mixtures health risk assessment are reflected in the Agency's guidance materials. This document is a product of that Panel and is intended as a supplement to the 1986 Guidelines document.

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LIST OF ABBREVIATIONS

ACGIH	American Conference of Government Industrial Hygienists
AHH	Aryl Hydrocarbon Hydroxylase
ATSDR	Agency for Toxic Substances and Disease Registry
B[a]P	Benzo(a)pyrene
BINWOE	Binary Weight-of-Evidence
BMD	Benchmark Dose
CRAVE	Carcinogen Risk Assessment Verification Endeavor
ED_x	Effective Dose in x Percent of Test Animals
GSH	Glutathione
HI	Hazard Index
HQ	Hazard Quotient
IRIS	Integrated Risk Information System
LD_x	Lethal Dose in x Percent of Test Animals
LOAEL	Lowest-Observed-Adverse-Effect Level
MFO	Mixed Function Oxidase
MOAEL	Minimum-Observed-Adverse-Effect Level
MOE	Margins of Exposure
MT	Metallothionein
NAS	National Academy of Science
NOAEL	No-Observed-Adverse-Effect Level
NRC	National Research Council

LIST OF ABBREVIATIONS (con.t)

OSHA	Occupational Safety and Health Administration
PAH	Polycyclic Aromatic Hydrocarbon
PB-PK	Physiologically Based Pharmacokinetics
PB-PK/PD	Physiologically Based Pharmacokinetics and Pharmacodynamics
PCB	Polychlorinated Biphenyl
POM	Polycyclic Organic Material
RfC	Reference Concentration
RfD	Reference Dose
RPF	Relative Potency Factor
TEF	Toxicity Equivalence Factor
TEQ	2,3,7,8-TCDD Toxicity Equivalents
TOC	Total Organic Carbon
TTC	Toxicity-Specific Concentration
TTD	Target Organ Toxicity Dose
UF	Uncertainty Factor
WHO	World Health Organization
WOE	Weight-of-Evidence

1. INTRODUCTION

1.1. BACKGROUND

While some potential environmental hazards involve significant exposure to only a single compound, most instances of environmental contamination involve concurrent or sequential exposures to a mixture of compounds that may induce similar or dissimilar effects over exposure periods ranging from short-term to lifetime. For the purposes of this guidance document, mixtures will be defined as any combination of two or more chemical substances regardless of source or of spatial or temporal proximity that can influence the risk of chemical toxicity in the target population (U.S. EPA, 1986). In some instances, the mixtures are highly complex consisting of scores of compounds that are generated simultaneously as by-products from a single source or process (e.g., coke oven emissions and diesel exhaust). In other cases, complex mixtures of related compounds are produced as commercial products (e.g., PCBs, gasoline and pesticide formulations) and eventually released to the environment. Another category of mixtures consists of compounds, often unrelated chemically or commercially, that are placed in the same area for disposal or storage, and have the potential for combined exposure to humans. Multichemical exposures are ubiquitous, including air and soil pollution from municipal incinerators, leakage from hazardous waste facilities and uncontrolled waste sites, and drinking water containing chemical substances formed during disinfection.

To address concerns over health risks from multichemical exposures, the U.S. EPA issued *Guidelines for Health Risk from Exposure to Chemical Mixtures* in 1986 (U.S. EPA, 1986). Those Guidelines described broad concepts related to mixtures exposure and toxicity and included few specific procedures. In 1989, the U.S. EPA published guidance for the Superfund program on hazardous waste that gave practical steps for conducting a mixtures risk assessment (U.S.

EPA, 1989). Also in 1989, the U.S. EPA published the revised document on the use of Toxicity Equivalence Factors for characterizing health risks of the class of chemicals including the dibenzo-dioxins and dibenzofurans. In 1990, the U.S. EPA published a Technical Support Document to provide more detailed information on toxicity of whole mixtures and on toxicologic interactions (e.g., synergism) between chemicals in a binary (two-chemical) mixture (U.S. EPA, 1990). The concept of toxicologic similarity was also discussed.

As more waste sites were evaluated for mixtures risks, it became apparent that the exposure scenarios for these sites were extremely diverse. Moreover, the quality and quantity of pertinent information available for risk assessment varied considerably for different mixtures. Such difficulties continue. Occasionally, the chemical composition of a mixture is well characterized, levels of exposure to the population are known, and detailed toxicologic data on the mixture are available. Most frequently, some components of the mixture are unknown, exposure data are uncertain or vary over time, and toxicologic data on the known components of the mixture are limited. Consequently, this document has been developed to supplement the earlier guidances and is organized according to the type of data available to the risk assessor, ranging from data rich to data poor situations. Procedures are described for assessment using data on the mixture of concern, data on a toxicologically related mixture, as well as data on the mixture component chemicals. The state of science varies dramatically for these three approaches. The whole mixture procedures are most advanced for assessing carcinogenic risk, mainly because of the long use of *in vitro* mutagenicity tests to indicate carcinogenic potency. *In vitro* test procedures for noncancer endpoints are still in the pioneering stage. In contrast, the component-based procedures, particularly those that incorporate information on toxicologic interactions, are most advanced for noncarcinogenic toxicity.

Mixtures risk assessments usually involve substantial uncertainties. If the mixture is treated as a single complex substance, these uncertainties range from inexact descriptions of exposure to inadequate toxicity information. When viewed as a simple collection of a few component chemicals, the uncertainties include the generally poor understanding of the magnitude and nature of toxicologic interactions, especially those interactions involving three or more chemicals. Because of these uncertainties, the assessment of health risk from chemical mixtures must include a thorough discussion of all assumptions and the identification when possible of the major sources of uncertainty. No single approach is recommended in this supplementary guidance. Instead, guidance is given for the use of several approaches depending on the nature and quality of the data.

1.2. OVERVIEW

The primary purpose of this document is to generate a consistent Agency approach for assessing health risks from exposures to multiple chemicals, denoted in this guidance by the general term, "mixtures." The resulting mixtures risk assessments are intended to assist decision makers by characterizing health risks for the particular exposure conditions of interest. Because exposure scenarios and the available supporting data are highly diverse, this document has been developed as a procedural guide that emphasizes broad underlying principles of the various science disciplines (environmental chemistry, toxicology, pharmacology, statistics) necessary for providing information on the relationship between multichemical exposure and potential health effects. Specific approaches to be used for the evaluation of the various kinds of mixture data are also discussed.

This document only addresses risks to human health from multichemical exposures. Ecological effects are beyond its scope, even though many of the procedures might be adaptable

to ecological risk assessment from multiple stressors. Because other Agency guidelines exist that address exposure assessment and specific toxic endpoint evaluations, this guidance focuses on procedures for dose-response assessment and risk characterization.

It is not the intent of this guidance document to regulate any social or economic aspects concerning risk of injury to human health or the environment caused by exposure to a chemical agent(s). All such action is addressed in specific statutes and federal legislation and is independent of this guidance.

This guidance document represents a supplement to the original Guidelines of 1986 and is intended to reflect the evolutionary scientific development in the area of chemical mixtures risk assessment. Consequently, many of the former discussions have been reiterated. New guidance has been provided that gives more specific details on the nature of the desired information and the procedures to use in analyzing the data. Among these are methods for using whole mixture data on a toxicologically similar mixture, methods for incorporating information on toxicologic interactions into a Hazard Index, procedures for including carcinogen interactions in a mixture risk characterization, and generalized procedures for mixtures involving classes of similar chemicals. There are also expanded discussions of the concerns when using only whole mixture data as well as when using only data on the individual chemical components.

The assessment of chemical mixtures is an area of active scientific investigation. Many of the procedures herein for chemical mixtures have had little or no application to date in actual health risk assessments. Their use is encouraged, along with research on new procedures to improve or replace those discussed here. As new information relevant to health risk from exposure to chemical mixtures becomes available, additional guidance documents will be published.

2. APPROACH TO RISK ASSESSMENT OF CHEMICAL MIXTURES

2.1. THE RISK ASSESSMENT PARADIGM FOR MIXTURES

Human health risk assessments done by U.S. EPA follow the paradigm established by the National Academy of Sciences (NRC, 1983). This paradigm describes a series of interconnected steps including hazard identification, dose response assessment, exposure assessment and risk characterization.

Hazard identification uses available data on biological endpoints to determine if a material is likely to pose a hazard to human health. These data are also used to define the type of potential hazard; e.g., does the material induce tumor formation or act as a kidney toxicant.

In the *dose-response assessment,* data (most often animal studies and occasionally from human studies) are used to estimate the amount of material that may produce a given effect in humans. In this step, the risk assessor may calculate a quantitative dose-response relationship usable for low dose exposure often by applying mathematical models to the data.

The *exposure assessment* seeks to determine the extent to which a population is exposed to the material. Exposure assessment uses available data relevant to population exposure, such as emissions data, measurement of the material in environmental media, and biomarker information. Fate and transport of the material in the environment, routes of exposure and pharmacokinetics of the material once in the body may all be considered in the exposure assessment. Data limitations on the environmental concentrations of interest often necessitate the use of modeling to provide relevant estimates of exposure.

Risk characterization is the final step of the risk assessment process that evaluates assessments of human health and ecological effects, identifies human subpopulations or ecological species at elevated risk, assesses exposures from multiple environmental media and describes the

uncertainty and variability in these assessments. In March, 1995, the Administrator of U.S. EPA issued the *Policy for Risk Characterization at the U.S. Environmental Protection Agency* reaffirming the principles and guidance found in the Agency's 1992 policy *Guidance on Risk Characterization for Risk Managers and Risk Assessors*. The purpose of this policy statement was to ensure that critical information from each stage of a risk assessment be presented in a manner that provides for greater clarity, transparency, reasonableness, and consistency in risk assessments. Most of the 1995 *Policy for Risk Characterization at the U.S. EPA* was directed toward assessment of human health consequences of exposures to an agent. Key aspects of risk characterization identified in the 1995 *Policy for Risk Characterization at the U.S. EPA* include these: bridging risk assessment and risk management, discussing confidence and uncertainties and presenting several types of risk information. Another publication, *Science and Judgment in Risk Assessment* (NAS/NRC, 1994), emphasized that the goal of risk characterization is to provide understanding of the type and magnitude of potential adverse effects of an agent under the particular circumstances of its release.

U.S. EPA regularly publishes guidelines to provide for consistency of application and communication of risk assessment. Guidelines were published in 1986 in the following areas: exposure assessment, assessment of developmental effects, germ cell mutagenicity, carcinogenic effects and Guidelines for the assessment of chemical mixtures (U.S. EPA, 1986, 1987). The Risk Assessment Guidelines for Developmental Toxicity were revised in 1991, those for Exposure Assessment in 1992 and revised Cancer Risk Assessment Guidelines were proposed in 1996. Guidelines for assessment of male and female reproductive effects were published in 1996. Neurotoxicity Guidelines were published in 1998. All of the U.S. EPA guidelines for human health risk assessment incorporate the steps of the NAS paradigm.

For this supplemental guidance on the risk assessment of chemical mixtures, the four paradigm steps are also interrelated and will be found within the assessment techniques that are presented. For some methods described herein, assessment of dose-response relies both on decisions in the area of hazard identification and on assessment of potential human exposures. The use of pharmacokinetics data and models in particular differs from single chemical assessment where they are often part of the exposure assessment. For mixtures, the dominant mode of toxicologic interaction is the alteration of pharmacokinetic processes, which strongly depends on the exposure levels of the mixture chemicals. In this guidance, there has been no effort to categorize methods strictly or arbitrarily into one of the paradigm steps. The methods are organized instead according to the type of available data. In general, the risk characterization step takes into account both human health and ecological effects and also assesses exposures from multiple environmental media. This guidance focuses only on the human health risk assessment for chemical mixtures and does not attempt to describe methods that are multi-route.

2.2. PROCEDURE FOR SELECTING A RISK ASSESSMENT METHOD

2.2.1. Introduction. The 1986 *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986) recommend three approaches to quantitation of health risk for a chemical mixture, depending upon the type of available data. In the first approach, toxicity data on the mixture of concern are available; the quantitative risk assessment is done directly from these preferred data. In the second approach, when toxicity data are not available for the mixture of concern, the Guidelines recommend using toxicity data on a "sufficiently similar" mixture. If the mixture of concern and the proposed surrogate mixture are judged to be similar, then the quantitative risk assessment for the mixture of concern may be derived from health effects data on the similar mixture. Finally, the third approach is to evaluate the mixture through an analysis of its

components, e.g., using dose-addition for similarly acting chemicals and response-addition for independently acting chemicals. These procedures include a general assumption that interaction effects at low dose levels either do not occur at all or are small enough to be insignificant to the risk estimate. The Guidelines recommend the incorporation of interactions data when available, if not as part of the quantitative process, then as a qualitative evaluation of the risk.

No single approach is recommended in this guidance document. Instead, guidance is given for the use of several approaches depending on the nature and quality of the available data, the type of mixture, the type of assessment being made, the known toxic effects of the mixture or of its components, the toxicologic or structural similarity of a class of mixtures or of mixture components, and the nature of the environmental exposure. The approaches presented herein represent a mix of well known, routine methods with several newer, less well established techniques. As a group, they provide the risk assessor with a number of reasonable options for evaluating chemical mixtures risk.

2.2.2. Proposed Approach. Consistent and clear terminology is critical to the discussion of chemical mixtures risk assessment methodology. It is important, then, in reading this document that the reader use the definitions of terms as presented in Tables 2-1 and 2-2. These tables will help the reader articulate differences among classification terms that group chemicals according to the assumptions and requirements of the methodologies. Table 2-1 presents chemical mixtures definitions in terms of specific criteria including the complexity of the mixture, similarity of biologic activity, similarity of chemical structure or mixture composition, the environmental source of the mixture, toxic endpoint, etc. The major concerns for the risk assessor are whether the available data on are components or whole mixtures, whether the data

TABLE 2-1

Definitions of Chemical Mixtures

Chemical Mixture Any set of multiple chemical substances that may or may not be identifiable, regardless of their sources, that may jointly contribute to toxicity in the target population. May also be referred to as a "whole mixture" or as the "mixture of concern."
Components Single chemicals that make up a chemical mixture that may be further classified as systemic toxicants, carcinogens, or both.
Simple Mixture A mixture containing two or more identifiable components, but few enough that the mixture toxicity can be adequately characterized by a combination of the components' toxicities and the components' interactions.
Complex Mixture A mixture containing so many components that any estimation of its toxicity based on its components' toxicities contains too much uncertainty and error to be useful. The chemical composition may vary over time or with different conditions under which the mixture is produced. Complex mixture components may be generated simultaneously as by-products from a single source or process, intentionally produced as a commercial product, or may co-exist because of disposal practices. Risk assessments of complex mixtures are preferably based on toxicity and exposure data on the complete mixture. Gasoline is an example.
Similar Components Single chemicals that cause the same biologic activity or are expected to cause a type of biologic activity based on chemical structure. Evidence of similarity may include parallel log-probit dose-response curves and same mechanism of action or toxic endpoint. These components are expected to have comparable characteristics for fate, transport, physiologic processes and toxicity.
Similar Mixtures Mixtures that are slightly different, but are expected to have comparable characteristics for fate, transport, physiologic processes and toxicity. These mixtures may have the same components but in slightly different proportions, or have most components in nearly the same proportions with only a few different (more or fewer) components. Similar mixtures cause the same biologic activity or are expected to cause the same type of biologic activity due to chemical composition. Similar mixtures act by the same mechanism of action or affect the same toxic endpoint. Diesel exhausts from different engines are an example.
Chemical Classes Groups of components that are similar in chemical structure and biologic activity, and that frequently occur together in environmental samples, usually because they are generated by the same commercial process. The composition of these mixtures is often well controlled, so that the mixture can be treated as a single chemical. Dibenzo-dioxins are an example.

TABLE 2-2

Definitions of Toxicologic Interactions between Chemicals*

Additivity When the "effect" of the combination is estimated by the sum of the exposure levels or the effects of the individual chemicals. The terms "effect" and "sum" must be explicitly defined. Effect may refer to the measured response or the incidence of adversely affected animals. The sum may be a weighted sum (see "dose addition") or a conditional sum (see "response addition").
Antagonism When the effect of the combination is less than that suggested by the component toxic effects. Antagonism must be defined in the context of the definition of "no interaction", which is usually dose or response addition.
Chemical Antagonism When a reaction between the chemicals has occurred and a new chemical is formed. The toxic effect produced is less than that suggested by the component toxic effects.
Chemical Synergism When a reaction between the chemicals has occurred and a different chemical is formed. The toxic effect produced is greater than that suggested by the component toxic effects, and may be different from effects produced by either chemical by itself.
Complex Interaction When three or more compounds combined produce an interaction that cannot be assessed according to the other interaction definitions.
Dose Additivity When the effect of the combination is the effect expected from the equivalent dose of an index chemical. The equivalent dose is the sum of component doses scaled by their potency relative to the index chemical.
Index Chemical The chemical selected as the basis for standardization of toxicity of components in a mixture. The index chemical must have a clearly defined dose-response relationship.
Inhibition When one substance does not have a toxic effect on a certain organ system, but when added to a toxic chemical, it makes the latter less toxic.
Masking When the compounds produce opposite or functionally competing effects at the same site or sites, so that the effects produced by the combination are less than suggested by the component toxic effects.
No Apparent Influence When one substance does not have a toxic effect on a certain organ or system, and when added to a toxic chemical, it has no influence, positive or negative, on the toxicity of the latter chemical.

TABLE 2-2 (cont.)
No Observed Interaction When neither compound by itself produces an effect, and no effect is seen when they are administered together.
Potentiation When one substance does not have a toxic effect on a certain organ or system, but when added to a toxic chemical, it makes the latter more toxic.
Response Additivity When the response (rate, incidence, risk or probability) of effects from the combination is equal to the conditional sum of component responses as defined by the formula for the sum of independent event probabilities.
Synergism When the effect of the combination is greater than that suggested by the component toxic effects. Synergism must be defined in the context of the definition of "no interaction", which is usually dose or response addition.
Unable to Assess Effect cannot be placed in one of the above classifications. Common reasons include lack of proper control groups, lack of statistical significance, and poor, inconsistent or inconclusive data.

*Based on definitions in U.S. EPA (1990). These definitions of interaction refer to the influence on observed toxicity, without regard to the actual mechanisms of interaction.

are composed of either similar components or similar mixtures that can be thought of as acting by similar toxicologic processes, or whether the data may be grouped into classes by emissions source, chemical structure or biologic activity. Table 2-2 provides definitions for terms that are used to describe various types of toxicologic interactions including forms of additivity, antagonism, synergism and other toxicologic phenomena. Tables 2-1 and 2-2 can be used by the risk assessor to classify available toxicity and exposure data in order to choose from among the risk assessment methods for chemical mixtures.

The proposed approach for the selection of a methodology for assessment of a chemical mixture is outlined in the flow chart shown in Figure 2-1, which begins with an assessment of data quality and then asks a series of questions that lead the risk assessor to selection of a method. The first major distinction that must be addressed is whether the type of available data is whole mixture data or mixture component information. This distinction leads the risk assessor towards methods that are available for these specific types of data and are not interchangeable. Methods available for whole mixtures are then dependent on whether there is information directly available on the mixture of concern or only on similar mixtures. Methods available for component data are then dependent on whether there are interactions data available, whether the components act with a similar mode of action, or whether the components can be thought of as belonging to a chemical class. In all cases, the outcome is either a quantitative or a qualitative assessment with a complete risk characterization presented. Figure 2-1 is deceptively simple, however, as many of the issues that are represented in the diagram require the use of scientific judgment or data that may not be readily available.

Table 2-3 presents a classification scheme for assessing the quality and nature of the available mixtures data. Consideration of the factors presented in Table 2-3 can be used to guide

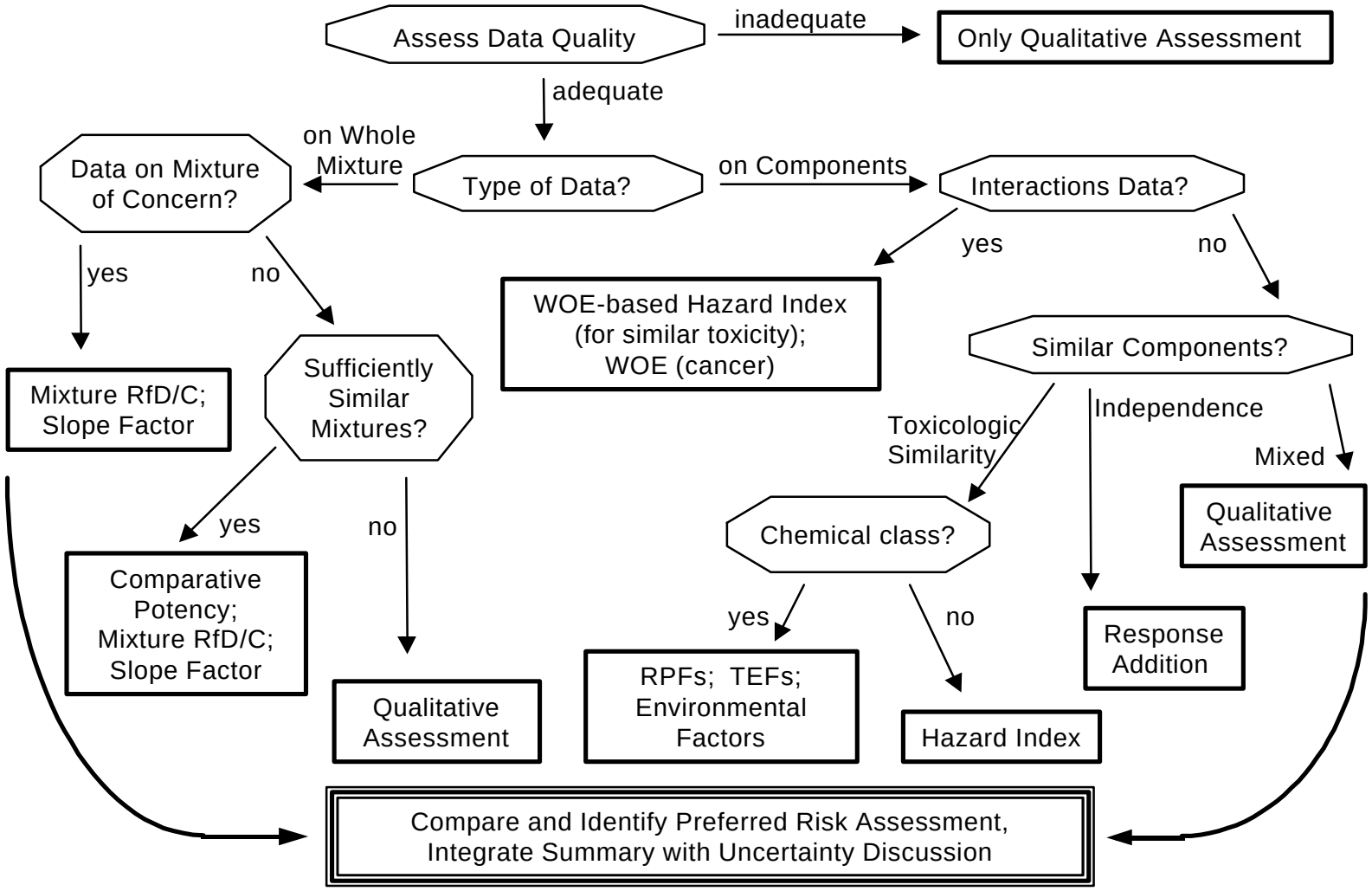


FIGURE 2-1
The different types of mixtures assessments based on the availability and quality of the data.
All possible assessment paths ahouls be performed.

TABLE 2-3

Classification Scheme for the Quality of Available Mixtures Data[a]

Exposure Information[b]

GOOD

- **S** Monitoring information either alone or in combination with modeling information is sufficient to accurately characterize human exposure to the mixture or its components.
- **S** Modeling information is sufficient to reasonably characterize human exposure to the mixture or its components.
- **S** Exposure estimates for some components are lacking, uncertain, or variable. Information on health effects or environmental chemistry suggest that this limitation is not likely to substantially affect the risk assessment.
- **S** Not all components in the mixture have been identified or levels of exposure are highly uncertain or variable. Information on health effects or environmental chemistry is not sufficient to assess the effect of this limitation on the risk assessment.

POOR

- **S** The available exposure information is insufficient for conducting a risk assessment.

Health Effects Information

GOOD

- **S** Full health effects data are available and relatively minor extrapolation is required.
- **S** Full health effects data are available but extensive extrapolation is required for route or duration of exposure or for species differences. These extrapolations are supported by pharmacokinetic considerations, empirical observations, or other relevant information.
- **S** Full health effects data are available, but extensive extrapolation is required for route or duration of exposure or for species differences. These extrapolations are not directly supported by the information available.
- **S** Certain important health effects data are lacking and extensive extrapolations are required for route or duration of exposure or for species differences.

POOR

- **S** A lack of health effects information on the mixture and its components in the mixture precludes a quantitative risk assessment.

Information on Interactions

GOOD

- **S** Assessment is based on toxicologic data on the mixture of concern.
- **S** Assessment is based on data on a sufficiently similar mixture.
- **S** Quantitative interactions of all components are well characterized.
- **S** The assumption of additivity is justified based on the nature of the health effects and on the number of component compounds.

POOR

- **S** Interactions information is inadequate, an assumption of additivity cannot be justified, and no quantitative risk assessment can be conducted.

[a]See text for discussion of sufficient similarity, adequacy of data, and justification for additivity assumptions.

[b]See the Agency's guidelines for exposure assessment (U.S. EPA, 1992) for more complete information on performing exposure assessments and evaluating the quality of exposure data.

the risk assessor through the steps in Figure 2-1. For example, a "GOOD" classification for each of exposure information, health effects information and information on interactions, would lead the risk assessor to consider the data quality to be adequate with good data available for both exposure and toxicity on the mixture of concern. Figure 2-1 would then guide the risk assessor to perform a risk assessment directly on the mixture of concern by calculating, for example, a mixture RfD or slope factor. A "POOR" classification for one or more of these categories would likely lead the risk assessor to decide that data quality was inadequate; in this case Figure 2-1 directs the risk assessor to perform only a qualitative risk assessment. With "fairly good quality" exposure information and health effects information but only "medium quality" information on interactions (component based, but quantitative interactions data), the risk assessor would conclude that data quality was adequate to estimate both the exposure and toxicity of the components of the mixture and furthermore to use the available interactions data in the assessment. Under these conditions, Figure 2-1 indicates that a weight-of-evidence (WOE) approach should be undertaken as appropriate for either carcinogenicity or systemic toxicity.

Tables 2-4 and 2-5 present summary information for use in selection of a chemical mixtures risk assessment method for whole mixtures data or component data, respectively. The entries in these tables are arranged by the type of available data and then by the objective of the risk assessment that is to be undertaken. The major difference in objective is whether a dose-response assessment is being made of the mixture (e.g., a cancer slope factor) or whether a risk characterization is being developed that combines both dose-response and exposure data to express health risk from the mixture (e.g., a hazard index). The features of each method in the tables are presented in terms of type of available data, type of assessment, toxic endpoints of concern, limits on the complexity of the mixture, applicability and requirements of the method,

uncertainties and assumptions, and a basic strategy for applying the method. Note that under the "type of assessment," the status of the method is reported in terms of how new or established it is. Each of these methods is presented in greater detail later in this guidance.

2.2.3. Defaults. The development of a risk assessment for a chemical mixture will generally involve the examination of complex exposures and toxicities and the application of specific methods as well as scientific judgment. This process necessarily involves a thorough examination and discussion of the uncertainties, limitations and assumptions inherent in exposure assessment, fate and transport, uptake and pharmacokinetics, and the magnitude and nature of toxicity and toxicant interactions. Because of the complexity of considerations that must be undertaken to develop a chemical mixtures health risk assessment, it is not practical to recommend a clear listing of default procedures that covers all cases.

For low exposure levels when no interactions information is available, default methods using an additivity assumption are given. For the component chemicals in a mixture that show dissimilar toxicity, response addition (Section 5.1) is recommended. For the component chemicals that show similar toxicity, dose addition (sections 4.0 and 6.0) is recommended. Under dose-addition, the general procedure is to scale the doses of the components for potency and add the doses together; the mixtures response is then estimated for the combined mixtures dose. Under response-addition, the general procedure is to first determine the risks per the exposure for the individual components; the mixtures risk is then estimated by adding the individual risks together. These processes are fundamentally different and require different assumptions of the data in order for them to be used appropriately (see Table 2-5 for specific procedures). Finally, if interactions data are available, the default recommendation is that they be incorporated into the risk

assessment either by using the interactions based hazard index (Section 4.4.2) or by including a qualitative assessment of the direction and magnitude of the impact of the interaction data.

Dose-addition is the default approach in situations where the dose for each individual component is at a level at which effects are not expected to occur, be observable, or be of concern; however, when the doses are combined, effects of concern are then expected or observed in response to the higher dose level of the mixture. A method based on dose-addition that has been used most often by the U.S. EPA is the Hazard Index (HI), where HI < 1 indicates a mixture exposure of no significant concern (U.S. EPA, 1989). True dose-addition is applied by scaling the potencies of all the components in the mixture with the same mechanism of action to an index chemical, adding the scaled doses together to give the equivalent dose in terms of the index chemical, and using the index chemical's dose-response curve to estimate the response for the equivalent total mixture dose. Dose-addition is different from response-addition because two assumptions are made: that all of the components have similar uptake, pharmacokinetics and toxicologic processes, and that the (log probit) dose-response curves of the components are parallel (Teuschler and Hertzberg, 1995). This means that, for equal effects, the dose of one component is a constant multiple of the dose of a second component.

The interaction based hazard index is the default approach for using interactions data to modify simple dose-addition. This approach uses binary interactions data for the components of the mixture to modify the HI. The factors that are used include the interaction magnitude at low doses, the toxicity of each component relative to each other component, the weight-of-evidence of the interactions data, and the relative proportions of the components in the mixture. The default approach for cancer interactions data is to provide a qualitative discussion of the effect of these data on the outcome of the mixtures risk assessment under response addition.

Response-addition is the default approach when the component chemicals are functionally independent. It is most often applied when an effect that is of concern is expected to be present at low dose levels for each of the component chemicals, even though it is highly unlikely to be observable at these low levels in either epidemiologic or toxicologic studies; the mixture risk is then the sum of the individually low risks of the independently acting component chemicals. For example, response-addition has often been used for the risk assessment of mixtures of carcinogens (Gaylor et al., 1997; U.S. EPA, 1989). Response-addition is different from dose-addition in that it does not assume similar kinetics or a similar mode of action and does not assume parallel dose-response curves. It assumes that the components of the mixture are considered to be functionally independent of one another at low exposure levels (Mumtaz and Hertzberg, 1993), so that the risks may be added together. Because response-addition does not require a similar mode of action across the chemicals in the mixture, it allows for combining risks across different types of endpoints.

2.3. DATA AVAILABLE ON THE MIXTURE OF CONCERN

For predicting the effects of subchronic or chronic exposure to mixtures, the preferred approach is to use subchronic or chronic health effects data on the mixture of concern and adopt procedures similar to those used for single compounds, either systemic toxicants or carcinogens (see U.S. EPA, 1987, 1989, 1996). Exposure and toxicity data on the mixture of concern are most likely to be available on highly complex mixtures (see Table 2-1) such as coke oven emissions, which are generated in large quantities and associated with or suspected of causing adverse health effects. Some of the issues that need to be considered include stability and bioavailability of the mixture in the environment, variability of the mixture composition over time, sources of the mixture, potential differences between mixtures tested in the laboratory and those

in the environment, and the need for specialized dose-response models for mixtures data. Those factors must be taken into account or the confidence in and applicability of the risk assessment is diminished.

Toxicity data obtained from concentrates or extracts of the original mixture of concern may not be predictive of human toxicity to the original mixture. Such data are more properly handled using procedures developed for toxicologically similar mixtures (Sections 2.4. and 3.2.).

2.4. DATA AVAILABLE ON SIMILAR MIXTURES

If the risk assessment is based on data from a mixture that is known to be generated with varying compositions depending on time or different emission sources, then the confidence in the applicability of the data to a risk assessment is diminished. This can be offset to some degree if data are available on several mixtures with the same components but with different component exposure levels, so that the likely range of compositional variation is covered. If such data are available, an attempt should be made to determine if significant and systematic differences exist among the chemical mixtures. If significant differences are noted, ranges of risk can be estimated based on the toxicologic data of the various mixtures. If no significant differences are noted, then a single risk assessment may be adequate, although the range of ratios of the components in the mixtures to which the risk assessment applies should also be given.

2.4.1. Criteria for Sufficient Similarity. If adequate data are not available on the mixtures of concern, but health effects data are available on a similar mixture (defined below), a decision must be made whether the mixture on which health effects data are available is or is not "sufficiently similar" to the mixture of concern to permit a risk assessment. The determination of "sufficient similarity" must be made on a case-by-case basis, considering not only the uncertainties associated

with using data on a dissimilar mixture but also the uncertainties of using other approaches such as dose additivity of the component chemicals.

A mixture is a candidate for toxicologic similarity if it has: the same components but in slightly different ratios; several common components but lacks one or more components; one or more additional components when compared to the mixture of concern. This judgment can be based on empirical measurements or on indirect evidence. The risk assessor must be able to support the assumption of toxicologic similarity and can do so by using any of a number of approaches: 1) establishing that a common mode of action exists across the mixtures or their components; 2) showing consistency in results of short-term screening assays; 3) distinguishing chemical class or chemical structure similarity; 4) identifying common components across the mixtures in similar proportions; 5) establishing a common source of formation or emission for the group of mixtures; and 6) applying statistical criteria for similarity (see Section 3.2.3.1 for a discussion of these ideas relative to the Comparative Potency approach). In determining reasonable similarity, consideration should be given to any information on the components that differ or are contained in markedly different proportions. In addition, if information exists on differences in environmental fate, in uptake and pharmacokinetics, in bioavailability or in toxicologic effects, it should be discussed. If such information is not available, it should be identified as a source of uncertainty. If toxicity data for the candidate mixture are only available for a different exposure route than the environmental route being addressed, extreme care should be used to ensure that the results are applicable, and that any effects restricted to the portal of entry to the body are appropriately discounted.

2.4.2. Uncertainties with Whole Mixture Studies. Even if a risk assessment can be made using data on the mixtures of concern or a sufficiently similar mixture, it may be desirable to

conduct a risk assessment based on toxicity data on the components in the mixture using procedures outlined in Section 2.2. When a mixture contains component chemicals whose critical effects are of major concern, e.g., cancer or developmental toxicity, an approach based on the mixture data alone may not be sufficiently protective in all cases. For example, the whole mixture approach for a two-chemical mixture of one carcinogen and one toxicant would use toxicity data on the mixture of the two compounds. However, in a chronic study of such a mixture, the presence of the toxicant could mask the activity of the carcinogen. That is to say, at doses of the mixture sufficient to induce a carcinogenic effect, the toxicant could induce mortality so that at the maximum tolerated dose of the mixture, no carcinogenic effect could be observed. Since carcinogenicity is generally considered by the Agency to be an effect of concern even at extremely low doses, it may not be prudent to conclude that the lack of a carcinogenic effect from such a bioassay indicates the absence of cancer risk at lower doses. (The type of carcinogenic effect is also a factor here; for example, low doses of a promoter are generally less of a concern than of a genotoxic carcinogen.) Consequently, the mixture approach should be modified to allow the risk assessor to evaluate the potential for masking, of one effect by another, on a case-by-case basis.

For most noncarcinogenic effects, reduced exposure levels lead to reduced severity of the effects. Carcinogenic effects have traditionally been assumed by EPA to be potentially fatal, so that reducing the exposure only lowers the expected response rate; the effect severity remains high. Environmental exposures, even at lower levels than those in the study, to a mixture with a known carcinogenic component then may pose a cancer risk in spite of negative results from a whole mixture study. Another example is a whole mixture assay that did not show developmental effects. Any developmental toxicity is considered an effect of major concern. If a component chemical is a known developmental toxicant, then the whole mixture data must be carefully

reviewed for a possible lack of statistical power or toxicologic sensitivity. Environmental exposures to such a mixture may then pose a risk of developmental toxicity in spite of the lack of developmental effects in the whole mixture study. In such cases, the uncertainty caused by the known effects of the component chemicals must be discussed. Additional evaluation may be warranted before developing the risk characterization.

2.5. DATA AVAILABLE ON MIXTURE COMPONENTS

2.5.1. Introduction to Additivity and Interaction Effects. If data are not available on an identical or reasonably similar mixture, the risk assessment may be based on the toxic or carcinogenic properties of the components in the mixture. When quantitative information on toxicologic interaction exists, even if only on chemical pairs, it should be incorporated into the component-based approach. When there is no adequate interactions information, dose or risk additive models are recommended. Several studies have demonstrated that dose (or concentration) addition often predicts reasonably well the toxicities of mixtures composed of a substantial variety of both similar and dissimilar compounds (Pozzani et al., 1959; Smyth et al., 1969, 1970; Murphy, 1980; Ikeda, 1988; Feron et al., 1995), although exceptions have been noted. For example, Feron et al. (1995) discuss studies where even at the same target organ (the nose), differences in mode of action led to other than dose-additive response. The assessment of multiple toxicant exposure has been addressed by the American Conference of Governmental Industrial Hygienists (ACGIH, 1983), the Occupational Safety and Health Administration (OSHA, 1983), the World Health Organization (WHO, 1981), and the National Research Council (NRC, 1980a,b). Although the focus and purpose of each group was somewhat different, all of the recommended approaches included some type of dose-additive model. Nonetheless, as discussed in later sections, dose-additive models may not be the most biologically plausible

approach if the compounds do not have the same mode of toxicologic action. Consequently, depending on the nature of the risk assessment and the available information on modes of action and patterns of joint action, the most reasonable model should be used.

2.5.2. Criteria for Dose-Addition vs. Response-Addition. Toxicologic interactions are defined in this guidance document (Table 2-2) to facilitate the selection and application of specific risk assessment methods. When adequate evidence for toxicologic interactions is not available, the no interaction approach (dose addition or response addition, as detailed below) will be employed. Toxicologic "interactions" are then operationally defined by data showing significant deviations from the "no interaction" prediction.

Several differing definitions of "no interaction" are discussed in the scientific literature. Plaa and Vénzina (1990) provide a nice historical overview of the differences in definitions, and Kodell and Pounds (1992) discuss some of the implications of these differences. Muska and Weber (1977) introduced the terms "concentration-addition" and "response-addition." Their definitions are based on ideas related to general toxicologic mechanisms; i.e., concentration addition (also termed dose addition) applies when the components act on similar biological systems and elicit a common response, whereas response addition applies when components act on different systems or produce effects that do not influence each other.

In this guidance, "no interaction" is defined using the two common concepts of Muska and Weber (1977): dose addition and response addition. These definitions have been selected because the underlying concepts are simple and because hypothesis tests exist to determine whether data are consistent with each of these concepts (see Gennings et al., 1995; Gennings and Carter, 1997). These definitions do not indicate specific toxicologic mechanisms, although they should be consistent with the major examples and concepts of toxicologic interaction. The risk assessment

using component data should then begin by selecting the most appropriate concept for the chemicals in the mixture. There will be many cases where the information does not support either dose or response addition. In those cases, the mixture should be further investigated, and consideration should be given to using methods that incorporate toxicologic interactions. Information on interactions can then be included as modifications of the "no interaction" approach that was selected (see Sections 4.4 and 5.2).

The primary criterion for choosing from dose or response addition as the no interaction approach is the similarity between the chemicals in the mixture. This judgmental decision should be based on information about the toxicologic and physiological processes involved, the single chemical dose-response relationships, and the type of response data available. To facilitate understanding, the discussions that follow will initially consider only two-chemical mixtures. For additional explanation of these concepts, see Svendsgaard and Hertzberg (1994).

2.5.2.1. Dose-Addition — In the simplest terms, two chemicals are dose additive if chemical 2 is functionally a clone of chemical 1. The chemicals are assumed to behave similarly in terms of the primary physiologic processes (uptake, metabolism, distribution, elimination) as well as the toxicologic processes. The mathematical definition of dose addition requires a constant proportionality between the effectiveness of the two chemicals. This means that, for equal effects, the dose of chemical 2 is a constant multiple of the dose of chemical 1. The dose-response functions are then congruent in shape. Let t be the proportionality constant that denotes the relative effectiveness of chemical 2 to chemical 1, often estimated by the ratio of their iso-effective doses, e.g., their ED_{10}s. Let r_1 and r_2 be response measures and f(d) and g(d) be the dose-response functions for chemicals 1 and 2, respectively. Then for doses d_1 and d_2 of chemicals 1 and 2 we have:

$$r_1 = f(d_1), \quad (2\text{-}1)$$

$$r_2 = g(d_2) = f(t*d_2) \quad (2\text{-}2)$$

The last equation (2-2) illustrates dose addition by converting dose d_2 into an equivalent dose of chemical 1 and then using the dose-response function f of chemical 1 to predict the response. For a mixture of the two chemicals, the mixture response r_m is then given in terms of the equivalent dose and dose-response function for chemical 1:

$$r_m = f(d_1 + t*d_2) \quad (2\text{-}3)$$

Among the many ways to decide dose-addition, the *isobole* is one of the more common graphical methods (see Figure 2-2). The isobole for a two chemical mixture is the graph of the various combinations of doses (d_1, d_2) at which a fixed response is observed (Gessner, 1995). In other words, the x-coordinate is the dose of chemical 1 and the y-coordinate is the dose of chemical 2 such that the joint exposure (d_1, d_2) produces the fixed response. This means that for all points plotted on the isobole, the same response occurs. For example, in Figure 2-2, the straight-line isobole represents the mixture doses in mg/kg that elicit a 10% response in the test animals. If a point, say (2000,50), is on the isobole, then the dose combination of 2000 mg/kg of chemical 1 and 50 mg/kg of chemical 2 will yield a 10% response in the test animals.

When the set of equal-response points is a straight line, the two chemicals are said to be dose additive. Deciding whether the points are linear is often judgmental, but statistical methods also exist to help make this determination (Gennings, 1995). Note that in the simple "clone" definition of dose addition, all isoboles for different response rates will be parallel. Other more

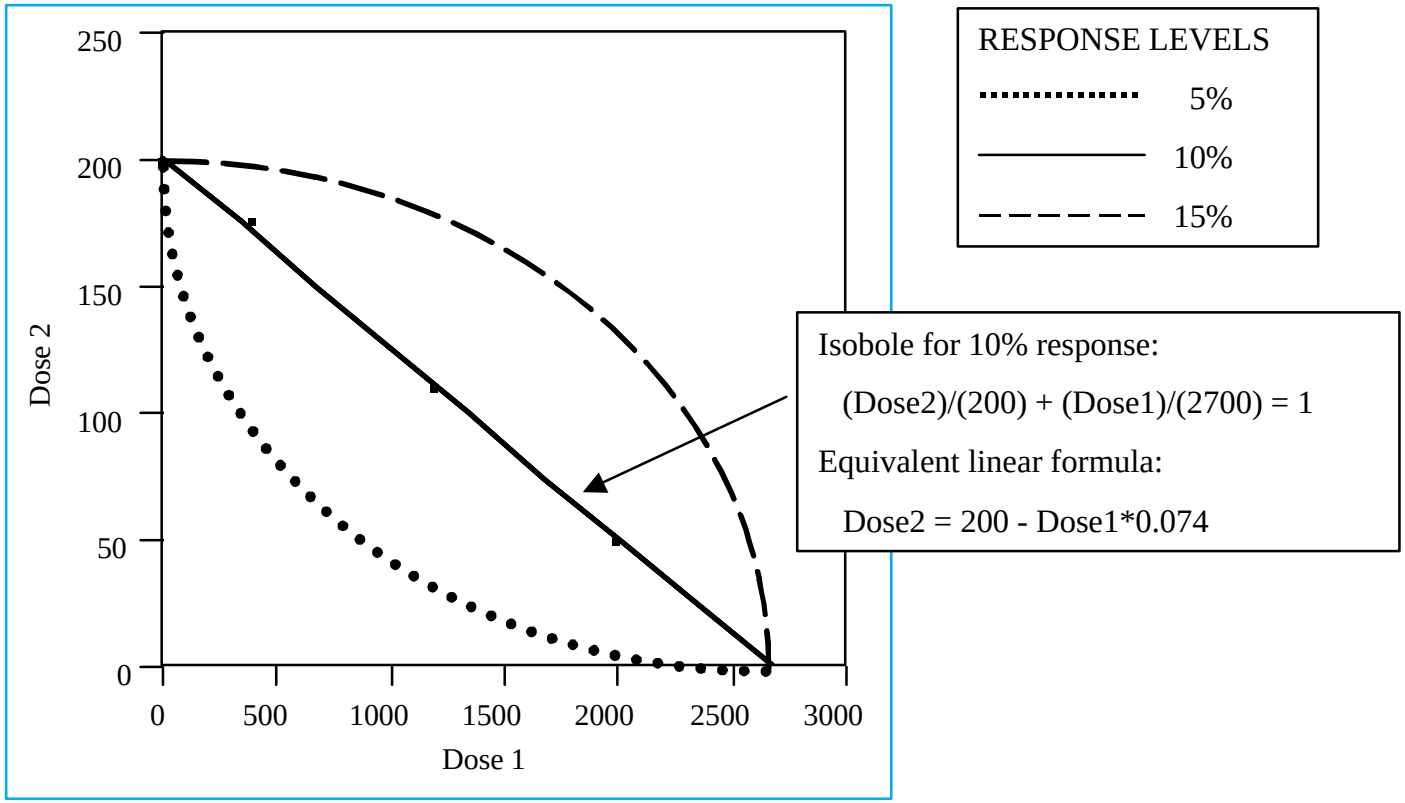


FIGURE 2-2

Isoboles for Three Response Levels of Combination Doses (d1, d2) of Two Chemicals

general definitions of dose addition have also been proposed (Svendsgaard and Greco, 1995), primarily where the lines for different response rates are linear, but not parallel (Svendsgaard and Hertzberg, 1994). When reviewing the literature for evidence supporting dose addition, the assessor should ensure that the definitions and assumptions are consistent with those used in this document. Foremost is that the isoboles should be linear. Second, unless the isoboles for a wide range of response levels are all parallel, the reported dose combinations used in generating the isobole should be comparable to the environmental doses being assessed. If the published isoboles relate to high doses, particularly those associated with unacceptable response levels (e.g., LD_{10}s), then justification must be given for assuming dose addition at lower environmental levels.

Recent work has demonstrated the issues that must be considered when assuming dose addition (Feron et al., 1995). Feron and colleagues tested various simple mixtures (n=4 or 9 components) at levels near the no-observed-adverse-effect levels (NOAELs). Studies in their laboratory on mixtures of chemicals with different target organs, or same target organ but different toxicity mechanisms, showed interactions when chemicals were at their minimum-observed-adverse-effect levels (MOAELs), and no effects when component chemicals were at 1/10 or 1/3 their respective NOAELs. Mixtures of chemicals with the same target organ (kidney) and similar toxic mechanisms showed consistency with dose addition when each chemical was at or slightly below its NOAEL. Similarity of toxic mechanisms is then stronger support for dose addition than is similarity of target organs. When exposures are near the NOAELs of the components, target organ similarity seems to be sufficient justification for dose addition.

Three component methods are discussed in this document that are based on dose addition: the Relative Potency Factor (RPF) method, the Toxicity Equivalence Factor method, which is a special case of the RPF method, and the Hazard Index method. They differ in the required

knowledge about toxic mechanism and in the extent over which toxicologic similarity is assumed. In each method, the exposure levels are added after being multiplied by a scaling factor that accounts for differences in toxicologic potency (also called toxic strength or activity).

The RPF method uses empirically derived scaling factors that are based on toxicity studies of the effect and exposure conditions of interest in the assessment. When extensive mechanistic information shows that all the toxic effects of concern share a common mode of action, then one scaling factor is derived for each chemical that represents all toxic effects, and all exposure conditions. This special case is the TEF method, where actual toxicologic equivalence between the component chemicals is assumed once the scaling factor is applied. When data are conflicting or missing, or indicate that different modes of action may aply to different effects or exposure conditions, separate factors may be derived for each effect or exposure condition, which are distinguished from the special TEFs by being called RPFs. In the general RPF and specific TEF methods, the scaling factor represents the toxicity relative to the toxicity of one of the chemical components, called the index chemical, which is usually the best studied chemical. The mixture exposure, given by the sum of the scaled exposure levels, is then the equivalent exposure in terms of the index chemical. The risk assessment then compares the equivalent index chemical exposure to that chemical's dose-response curve.

The Hazard Index method has weaker assumptions and data requirements, is more generally applicable, and has more uncertainty in the resulting assessment. Instead of requiring knowledge of similar mode of action, the HI method only requires similarity in target organ. As with the general RPF method, a separate HI is determined for each target organ of concern. Instead of converting the component exposure levels into an equivalent index chemical exposure, the scaling factors are standardized so that the resulting sum is dimensionless, and the HI is

interpreted by whether or not it is greater than 1. The scaling factors for the Hazard Index are only based on each component's toxicity, preferably related to the target organ being assessed so that the interpretation of the HI value can be tied to the target organ risk. For example, if the liver effects ED10 is used (so that 1/ED10 is used as the toxic potency factor), then when HI=1, the mixture is at its ED10 for liver toxicity. Similarly, if some estimate of a practical threshold exists for each component, then HI=1 indicates that the mixture is at its practical threshold. The scaling factors for the HI method should then be defined so that the resulting interpretation of HI=1 allows a clear risk assessment interpretation for the mixture. In previous EPA applications of the HI method, the HI has served only as a decision index, where HI>1 leads to more investigation or to remedial action. If enough information becomes available on the components to assume a similar toxic mode of action, then RPFs could be developed instead.

2.5.2.2. Response Addition — Under response addition, the chemicals are assumed to behave independently of one another, so that the body's response to the first chemical is the same whether or not the second chemical is present. In simplest terms, classical response addition is described by the statistical law of independent events, with "response" measured by the percentage of exposed animals that show toxicity. Using the same notation defined above for equations 2-1 through 2-3, the statistical law of independence is:

$$r_m = 1 - (1 - r_1)*(1 - r_2) \qquad (2\text{-}4)$$

In terms of mixture response, this equation says that the response to either chemical 1 or 2 is one minus the probability of not responding to either chemical. Expanding the right-hand-side, one obtains:

$$r_m = r_1 + r_2 - r_1*r_2 \qquad (2\text{-}5)$$

which, for small single chemical responses, is well approximated by the simple summation:

$$r_m = r_1 + r_2 \qquad (2\text{-}6)$$

Response addition has also been reported where "response" is a measured effect (Ikeda, 1988), but no publications have been located that explain this approach in any detail. The component effects are numerically added to give an estimated measured effect for the mixture. The simple summation implies that each component effect is small so that the effects caused by different components are not influenced by each other. Because this "effect addition" is not well characterized or investigated, this approach is not recommended at this time. Any risk assessment based on effect addition should be restricted to the specific effects and dose ranges given in the supporting studies.

Several variations of response addition have been developed (see U.S. EPA, 1986, Appendix A). Some of these variations require additional information and assumptions. When reviewing the literature for evidence supporting response-addition, the assessor should ensure that the definitions and assumptions are the same as those used in this document, or at least that the interpretations are consistent with the procedures in this guidance document.

2.5.2.3. Low Dose and Low Response Risk Assessments — One of the important differences between risk assessment for individual chemicals vs. a mixture assessment occurs when exposure levels are below the risk criteria values for the individual components of the mixture. The individual chemical assessments, performed separately, would conclude that none of the chemicals poses a significant risk. If the mixture contains several toxicologically similar chemicals with no evidence of interaction, then dose addition would be applied and the higher combined mixture dose could lead to an assessment of significant risk of toxic effects.

If the mixture contains only toxicologically dissimilar chemicals, then response addition would usually be applied because of the assumption of independent action. For example, if these chemicals have no-threshold, low-dose linear dose-response curves (as EPA has traditionally

assumed for carcinogens), then the risks can accumulate and be unacceptable. For example, 40 chemicals each posing $3x10^{-5}$ risk would give a mixture risk of $1x10^{-3.}$ If these chemicals have threshold-like or "hockey-stick" shaped dose-response curves, where doses below a certain value are associated with nonadverse effects, the mixture assessment may conclude no significant risk. This conclusion is plausible not only because of the very low percent response for each chemical, but also because the intensity of the effect decreases with dose. These cases show that the choice of dose addition vs. response addition, along with the nature of the toxicity, can be pivotal to the risk characterization.

Example. Consider an oral exposure to three chemicals, each close to but below its Reference Dose (RfD). The RfD is defined as a daily single chemical oral exposure level below which significant adverse effects are deemed unlikely to occur. (The analogous value for an inhalation exposure is called a Reference Concentration (RfC).) Let the exposure levels and RfDs of the chemicals be d_1=13, d_2=7, d3=22 and RfD_1=16, RfD_2=8, RfD_3=24, respectively, all in the same units. The individual chemical risks r_1, r_2 and r_3 are then negligible because the exposures are all less than the individual RfDs; an assessment of exposure to the chemicals evaluated individually would conclude that no significant risk exists. Let the dose-response function for chemical 1 be r_1=f(d) and assume the chemicals are toxicologically similar so that dose addition is applied, i.e., the concern is the cumulative dose of the three chemicals. Let the toxicity of chemical 2 be twice that of chemical 1 (so we define t_{21}=2) and chemical 3 be 2/3 that of chemical 1 (so we define t_{31}=0.7). Then the mixture response, r_m, under dose addition is:

$$r_m = f\,[d_1+(t_{21}*d_2) + (t_{31}*d_3)] = f\,[13+(2*7)+(0.7*22)] = f\,[42] \qquad (2\text{-}7)$$

The mixture exposure is now presented in terms of the equivalent level of chemical 1, and so can be interpreted as nearly three times the RfD (see Section 3.2 for a discussion of a mixture RfD).

If the three chemicals are functionally independent, then the concern is the cumulative response of the three chemicals. Adapting the independence equation (2-4) to an arbitrary number of chemicals, the mixture percent response is given by:

$$r_m = 1 - (1 - r_1)*(1 - r_2)*. . . *(1 - r_n) \quad (2\text{-}8)$$

For the case of three chemicals, the mixture percent response is given by:

$$r_m = 1 - (1 - r_1)*(1 - r_2)*(1 - r_3) \quad (2\text{-}9)$$

$$= r_1 + r_2 + r_3 - r_1*r_2 - r_1*r_3 - r_2*r_3 + r_1* r_2*r_3$$

For very small single chemical risks, all the cross-products are insignificant, resulting again in a simple summation. For three chemicals, each at a risk of 0.005, this simplification gives the mixture risk as: $r_m = 0.005 + 0.005 + 0.005 = 0.015$.

This simple sum can also be applied to more complex mixtures. For example, a mixture with 40 chemical components, each at a risk of 0.005, would have a true risk (equation 2-8) of 0.18, compared to the simple sum estimate of 0.20. The simple sum approximation is then fairly accurate.

The above discussions on response addition have emphasized the application to cases with small component responses. The methods may fail and give unrealistic estimates if individual chemical responses are not small. For example, with percent response, the cross-product terms must be included if the individual risks are not small because the response additive formula has an upper bound, i.e., the mixture response must be less than or equal to 1 (100%). If response is the measured effect then there are physiological limits to that measured quantity, another type of bound that could be upper or lower depending on the effect. This problem does not occur with dose addition; the same constraints on response are present, but are automatically met because the combined dose must follow the actual dose-response curve of one of the chemicals.

2.5.2.4. Evidence for Dose or Response Additivity — Several studies have been published that suggest dose or response additivity adequately characterize mixtures risk. In too many cases, however, the study was not designed properly for detecting departures from additivity (see U.S. EPA, 1990 for a survey and discussion of statistical methods in interaction studies) so generalizations of the commonness some kind of additivity are currently difficult to make. Some sense of the opinion of toxicologists, however, can be gained from some fairly recent publications, in which dose or response addition is recommended as a plausible default procedure or interpretation of "no interaction."

Ikeda (1988) surveyed the literature and found few cases, by his judgment, that showed "clear-cut cases of potentiation" and he concluded (p. 418): "Thus, the most practical approach in evaluating the combined effect of chemicals seems to be the assumption of additive effects." He also noted that assuming additivity of effects for chemicals with dissimilar modes of action is more protective than independence. Furthermore, except for their initial overview, Plaa and Vénzina (1990) focus on concentration (i.e., dose) addition. In contrast, response addition is rarely defined as a no-interaction approach in published studies on toxicologic interactions, and when it is mentioned, it is usually just stated to be a possible no-interaction model, without any motivating discussion. The NAS book (NAS, 1988, p.100) on complex mixtures notes that "no-interaction" in its Chapter 1 is dose addition, while in its discussion of ordinary linear statistical models, no-interaction refers to response addition. The original U.S. EPA guidelines for mixtures risk assessment (U.S. EPA, 1986) recommend default no-interaction approaches of dose addition for nongenotoxic toxicants acting by similar modes of action or affecting common organs, and response addition for carcinogenic risk.

The evidence for either response addition or dose addition is then not strong, and clearly is not comprehensive for the varying types of chemicals considered in environmental risk assessment. The choice of these two concepts as default "no interaction" definitions is then primarily based on clarity, simplicity and ease of implementation. Whenever evidence exists that clearly disagrees with both dose and response addition, then alternative approaches should be considered, such as those presented later that incorporate data on pairwise interactions.

2.5.3. Definitions of Toxicologic Interactions. Several quantitative descriptions of interaction have been proposed during the past 50 years. Plaa and Vénzina (1990) provide a historical overview of the differences in definitions, and Kodell and Pounds (1992) discuss some of the implications of these differences. One of the earliest quantitative characterizations of interactions was by Bliss (1939): similar joint action, independent joint action, and synergistic or antagonistic joint action. Plaa and Vénzina (1990) propose the terms *additive* (sum of individual effects, an admittedly vague definition), *infra-additive* and *supra-additive* as having the advantage of not requiring consideration of mechanisms. Table 2-2 recommends a set of definitions for use in chemical mixtures risk assessment. It clarifies the terminology related to additivity and interaction effects for both cancer and noncancer endpoints.

2.5.4. Risk Assessment Strategy. Approaches based on the mixture's chemical components are recommended for relatively simple, identified mixtures with approximately a dozen or fewer chemical constituents. For exposures at low doses with low component risks, the likelihood of significant interaction is usually considered to be low. Interaction arguments based on saturation of metabolic pathways or competition for cellular sites usually imply an increasing interaction effect with dose, so that the importance at low doses is probably small. The default component procedure at low exposure levels is then to assume response addition when the component

toxicological processes are assumed to act independently, and dose (or concentration) addition when the component toxicological processes are similar. For dose (concentration) addition, a specific Hazard Index procedure is recommended. For higher exposure levels, or when adequate data on interactions suggest other than dose or response additivity at low doses, such information must be incorporated into the assessment. Specific procedures are recommended for interactions based on the available data (Sections 4.4. and 5.2.).

2.5.5. Cautions and Uncertainties with Component-Based Assessments. The component-based procedures discussed earlier for dose-response assessment and risk characterization are intended only for simple mixtures of a dozen or so chemicals. The uncertainties and biases for even a small number of chemical components can be substantial. Component-based methods are particularly susceptible to misinterpretation because the listing of chemical components in a mixture is often misconstrued as implying a detailed understanding of the mixture toxicity and, by inference, the estimated mixture risk. The risk characterization must include a discussion of what is known as well as what is missing or poorly understood in order to convey a clear sense of quality and confidence in the risk assessment.

2.5.5.1. Exposure Uncertainties — The general uncertainties in estimating mixture exposure are addressed in the Agency's guidelines related to exposure assessment (U.S. EPA, 1992). The risk assessor should discuss these exposure uncertainties in terms of the strength of the evidence used to quantify the exposure. When appropriate, the assessor should also compare monitoring and modeling data and discuss any inconsistencies as a source of uncertainty. For mixtures, these uncertainties may be increased as the number of compounds of concern increases.

If levels of exposure to certain compounds known to be in the mixture are not available, but information on health effects and environmental persistence and transport suggest that these

compounds are not likely to be significant in affecting the toxicity of the mixture, then a risk assessment can be conducted based on the remaining compounds in the mixture, with appropriate caveats. If such an argument cannot be supported, no final risk assessment can be performed with high confidence until adequate monitoring data are available. As an interim procedure, a risk assessment may be conducted for those components in the mixture for which adequate exposure and health effects data are available. If the interim risk assessment does not suggest a hazard, there is still concern about the risk from such a mixture because not all components in the mixture have been considered.

In perhaps a worst case scenario, information may be lacking not only on health effects and levels of exposure, but also on the identity of some components of the mixture. Analogous to the procedure described in the previous paragraph, an interim risk assessment can be conducted on those components of the mixture for which adequate health effects and exposure information are available. If the risk is considered unacceptable, a conservative approach is to present the quantitative estimates of risk, along with appropriate qualifications regarding the incompleteness of the data. If no hazard is indicated by this partial assessment, those partial results should be conveyed to the risk manager, but the risk assessment should not be quantified until better health effects and monitoring data are available to adequately characterize the mixture exposure and potential hazards.

2.5.5.2. Dose-Response Uncertainties — For many simple mixtures for which a component-based approach might be applied, studies on interactions, even pairwise interactions, will be missing. Use of a dose or response additive model is easily implemented, but justification for such approaches is largely based on conceptual arguments, not empirical studies. An investigation into available interaction studies (U.S. EPA, 1990) found that roughly half of these

did not report any attempt at data analysis, or only reported significance levels ("p values") with no indication of the statistics used. As indicated previously, recent studies by Feron et al. (1995) show that there are exceptions to most rules regarding interactions, even the common assumption that additivity is acceptable if chemicals target the same organ. Recent studies on dose additivity have focused on very simple mixtures of chemically and metabolically similar chemicals (Gennings, 1997; Simmons et al., 1995). Improvements in experimental design and statistical hypothesis testing for dose additivity, along with better understanding of the chemical characteristics that accompany observed dose additivity, should lead to improved predictive ability and justification for dose-addition as a default approach.

Conclusions regarding toxicologic interaction are also weakly supported by empirical studies. Many studies (U.S. EPA, 1990) failed to identify what the "no-interaction" hypothesis was, so that any conclusions regarding nonadditive interaction were difficult to interpret. Other studies identified the no-interaction hypothesis, but employed incorrect experimental designs, so that the conclusions were not justified. Perhaps the most substantial weakness in the understanding of toxicologic interactions is the lack of studies, models and concepts for interactions involving more than two chemicals. The key assumption in both of the interaction weight-of-evidence methods (described in Section 4.4.) (Mumtaz and Durkin, 1992; Hertzberg, 1996) is that, at least for low doses, the resulting influence of all toxicologic interactions in a mixture is well approximated by the pairwise interactions. No studies have been located to date that investigate that assumption, although two studies are in progress at the U.S. EPA and ATSDR.

Toxicologic understanding of interaction is also limited. Although interaction mechanisms are commonly assumed to involve either pharmacokinetics and metabolism or toxicologic

receptors, nearly all studies on mechanisms and modes of interaction focus on pharmacokinetics (El-Masri et al., 1995). Current pharmacokinetic models for interactions usually address two- or three-chemical mixtures. Clearly, more research on complex interactions is necessary to improve risk assessment interactions information.

2.5.5.3. Presenting Component-Based Risk Characterization — The consequence of this early stage of mixtures risk research is that the risk assessor must use considerable judgment along with plausible approaches. The results, however, must be presented transparently. Although the procedures described in Chapters 4 and 5 are developed from available concepts and data on simple mixtures, all component-based quantitative mixtures risk assessments should be limited to one significant digit for the risk value, unless substantial justification is given for higher precision.

Mixtures composed of chemicals with RfDs or RfCs must be assessed and presented carefully. A common interpretation is that mixtures with few components, each less than its RfD or RfC, pose no significant risk. As discussed above, for toxicologically similar chemicals, this conclusion can be in error because the joint exposures contribute to the same potential toxicity and effectively represent a cumulative dose; thus a dose-additive assessment should be performed. For a mixture of a few dissimilar chemicals, where an assessment is based on response addition, the mixture risk would likely be judged negligible, particularly if the effects supporting the RfDs and RfCs are minor. When the toxic effects are of major concern, such as cancer or developmental toxicity, the estimated mixture risk should be judged in the context of the effects, the shapes of the dose-response curves, and the characteristics of the exposed population.

Whenever an assessment is based on component toxicity values, the risk characterization must discuss the quality of the individual chemical estimates that are used. RfDs and RfCs differ in

quality as reflected by the variation in their uncertainty factors and the confidence statements listed in the IRIS files. The cancer potency values also have uncertainty, as reflected by confidence levels and goodness-of-fit values when models are used, as well as by qualitative descriptors of the weight of evidence that the chemical is a human carcinogen. All these measures of uncertainty and unevenness of component estimates must be described, at least in summary fashion, in the risk characterization.

2.6. FUTURE DIRECTIONS

2.6.1. Overview. Risk assessment methods for chemical mixtures are progressing along paths similar to risk assessment for single chemicals, by incorporating more knowledge of specific modes of toxicologic action of the chemicals and by greater use of statistical methods and mathematical models. Where the field differs, however, is in the more extensive use of quantitative inference from tested chemicals to untested chemicals. Mixture exposures can be extremely varied, with differences in total dose, composition and relative proportions. Consequently, only a small fraction of environmental mixtures can actually be tested for dose-response characteristics. Two options then seem feasible: directly investigating a few high priority mixtures, and, for the remainder, developing extrapolation methods for using available data on the mixture components or on similar mixtures.

The first option requires priority setting, which for mixtures is its own research area. Once a few mixtures posing the highest risk have been identified, research should seek to evaluate their exposure, toxicity, and risk characteristics. Because even the highest priority mixtures are likely to pose complex and varied exposure possibilities, much of the research effort should involve developing highly efficient experimental designs and uncertainty methods so that several scenarios can be characterized for each mixture.

The second option, for addressing all the remaining mixtures, is to develop methods that can extrapolate exposure and toxicity estimates from available data. Such risk assessment methods should ideally be developed in conjunction with laboratory studies that are needed for validation. One example concerns interaction studies, such as those detailed in the EPA's Mixtox data base (Marnicio et al., 1991;U.S. EPA, 1990) of *in vivo* toxicologic interaction studies. In the Mixtox data base, 99% of the interaction evaluations involve only pairs of chemicals. Consequently, the interactions based Hazard Index (section 4) uses pairwise interactions to approximate the mixture response. The number of pairs studied to date, however, is a small fraction of the number of possible chemical combinations, and the number of whole mixtures studied is far smaller yet. For example, with a simple mixture of only 20 chemicals, there are 90 pairs, but over a million possible combinations (pairs, triples, etc.), thus the interest in extrapolating from pairwise interactions to the whole mixture. Because of this sparseness of existing data both on whole mixtures and on interactions, the accuracy of these extrapolation methods will be difficult to judge. The inferential procedures for mixtures risk are then likely to be adopted based on biological plausibility and on relatively few validation studies.

For either option, it is important to link the available information and the risk assessment method to the real world exposure. For example, most human exposures are to complex mixtures of multiple chemicals at low doses whose composition changes over time. Epidemiologic research is important to assess for the purposes of hazard identification, as well as characterization of exposure and dose-response whenever the data are adequate for quantification. These data can be used in conjunction with animal toxicity data to formulate appropriate, realistic risk estimates.

2.6.2. Desirable Tools and Risk Methods for Mixtures. The next phase of research in risk assessment for multichemical exposures should focus on the development of information and methods so that the resulting risk tools have certain desired characteristics. Paramount among these characteristics is that the tools are feasible to implement, both regarding time and resources, and are biologically plausible.

One focus area of research must be the evaluation and improvement of the methods proposed in this guidance document. The two that seem most amenable to modification based on new data include the interactions based Hazard Index for mixture components, and the comparative potency method for whole mixtures. The model used for the interaction based Hazard Index includes several factors that can be improved by new studies quantifying the magnitude of pairwise interaction as it depends on total dose and on the component proportions. The comparative potency method needs further demonstration with different kinds of mixtures than the PAH mixtures originally used and for endpoints other than cancer. It could also be improved by better statistical methods for estimating the cross-assay and cross-mixture proportionality constants. In all the proposed methods, there is the need for research into descriptions of uncertainty.

Research on new methods and tools should be oriented toward certain performance aspects related to mixtures risk assessment, not just improved knowledge about mixture fate or toxicity. The following items are suggested areas for research that have been identified from current risk research and actual mixture assessments. In most cases, the research should produce tools that are feasible: requiring a short time and low information resources to implement, and using existing data. The research areas are roughly grouped under the categories of priority lists, exposure and fate, and toxicity.

Priority lists:

- List of chemical pairs for toxicologic interactions testing.

  The ranking should include toxic potency, frequency of occurrence in the environment, and exposure level.

- List of complex mixtures for whole mixture toxicity testing.

  The ranking should consider toxic potency, the extent of population exposure, the availability of appropriate toxicity tests, and the constancy of composition of the environmental mixture over the time period covered by the regulatory action.

- List of chemicals and mixtures for degradation testing.

  The ranking should consider toxic potency, the extent of population exposure, the toxicity of the original or produced substances, and the availability of appropriate degradation tests. Degradation should also be studied by simulation with Quantitative Structure Activity Relationship (QSAR) models.

Exposure and fate:

- Identification methods that avoid masking by other chemicals.

  Of particular interest is the ability to separate close congeners from true background material (e.g., in GC/MS profiles), and the ability to detect a wide range of exposure levels in the same sample so that high toxicity/low concentration chemicals are not overlooked.

- Sampling strategies for hot spot monitoring where each spot has different driver chemicals.

  The emphasis should be on efficient strategies that ensure detection of the hot spots. The error and uncertainty should be characterized as the sample strategy changes.

- Influence of one chemical on the transport of another, including both environmental transport and uptake by humans.

  One example is vapor sorbed to particulates affecting airborne transport as well as the deposition pattern in the lung.

- Chemical interactions affecting bioavailability in the environmental medium or *in vivo*.

  One application is predicting the long term exposure to chemicals in soil.

- Procedures for artificial degradation or weathering of complex mixtures.

These procedures would allow direct toxicity estimates of degraded mixtures and promote the development of monitoring strategies for toxic degradation products.

- Investigation of natural and artificial attenuation of mixtures.

  Much work is needed to characterize the changes in mixtures from attenuation, regarding composition as well as toxicity. The potential for co-production of synergists during natural attenuation needs to be estimated and quantified. The prediction of long term continuation of attenuation processes, whether natural or human initiated. Of particular interest is whether short- term monitoring data showing attenuation progress can be used to estimate the long-term expected progress.

Toxicity:

- Validation of lab mixture as surrogate for the environmental mixture.

  The toxicity testing of the laboratory mixture, such as complex mixture extracts and fractions, has already led to new laboratory techniques. The next steps should evaluate the laboratory mixture toxicity for accuracy in reflecting the actual mixture's toxicity.

- Quantitative extrapolation models and risk estimation methods using existing data on a few similar chemicals (common mode of action) to extrapolate to untested chemicals.

  The simple dose addition methods now used are applied only to toxicologically similar chemicals where parallelism of dose-response curves is assumed. The models should be further explored for possible generalization and application to a wider group of chemicals. The criteria used to decide toxicologic similarity of a chemical class should be further refined, incorporating more information on the chemical class than just mode of action. For all the component methods, there is the need to quantify the expected variability in the predicted mixture response. Statistical methods including QSAR should be further developed for estimating toxicity of untested chemicals that are not part of a similarity class, and for estimating interaction magnitude based on interaction similarity analyses.

- Mathematical models of complex mixtures.

  Biologically based mathematical models of toxicity and interaction should be expanded to represent more complex mixtures with various combinations of different modes of action and combinations of different exposure time frames.

- Testing of real world exposures

Toxicologic studies in animals should be designed that attempt to closely simulate the actual human exposure, including intermittent low-dose exposures and multiple routes.The resulting animal data should be examined in conjunction with available epidemiologic and human clinical data when developing a risk assessment.

- Development of screening assays for mixtures

  Short-term screening assays are needed to screen mixtures for those combinations of chemicals that are most toxic or that potentially interact. Such screening assays can provide guidance on those mixtures that should be further studied in longer term sub-chronic or chronic animal bioassays. Examples assays include the medaka fish, FETAX (frog embyro), and various *in vitro* methods, including those that are using mammalian cells.

Once these research areas have been sufficiently investigated to produce feasible risk tools, then the testing of key chemicals must follow in order to build the data bases and data management software needed for the routine use of these tools. The second phase of the research should then focus on evaluation and refinement of these tools. As was found with the analysis of consistency of pairwise interactions (Durkin, et al., 1995), the evaluation of the mixtures risk tools may lead to research on new statistical and toxicologic methods.

3. METHODS FOR EVALUATING WHOLE MIXTURES

3.1. DOSE-RESPONSE ASSESSMENTS FOR WHOLE MIXTURES

3.1.1. Introduction. A dose-response assessment has been done by the Agency for several whole mixtures (see Sections 3.1.2 and 3.1.3 below). Under certain conditions, a dose-response assessment can be determined for the mixture itself; a major requirement is that the mixture composition be stable. This implies that for the exposure duration addressed by the risk assessment, the relative proportions of the mixture component chemicals are roughly constant so that the mixture can be treated as though it were a single chemical.

The use of such a dose-response estimate depends on whether the environmental mixture of concern and the mixture whose data is used to derive the dose-response assessment can be considered to be similar mixtures. This concept of "similar mixtures" can be viewed along a continuum beginning with exposure and dose-response data directly on the environmental mixture of concern (e.g., human data from an occupational study) to comparing a mixture for which laboratory dose-response data is available to an environmental mixture (e.g., animal toxicity data on a commercial mixture as compared with the same product that has chemically degraded to some degree in the environment). If the mixtures are highly similar, we would apply the dose-response assessment with high confidence. As the mixtures being compared become more dissimilar, there would be less confidence in applying a dose-response assessment because the dissimilar mixtures would have different components, or different concentrations of the same components, so that there would be a greater potential for different toxic effects to occur that would mask the toxic effect from the mixture of interest. Thus, the risk assessor should be able to apply dose-response assessments with confidence from highly similar mixtures, know the

problems of applying them from dissimilar mixtures, and make some judgment about where on this continuum each case lies.

For example, a dose-response assessment for a single chemical by an oral route of exposure may result in the calculation of an RfD, defined on the Agency's Integrated Risk Information System (IRIS) as follows (U.S. EPA, 1999):

> The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

The RfD is used for oral exposures. For inhalation exposures, the analogous value is the Reference Concentration (RfC). The RfD is based on the assumption that for a critical effect, such as cellular necrosis, there exists a dose level at which the effect is not observed, not expected to occur, or is at a level of severity that is not of concern (e.g., the effect is reversible or is a mild precursor effect). The mixture RfD is then given as a daily dose (e.g., mg/kg/day), where the mg exposure is for the mixture as a whole. The mixture RfD can be interpreted as an RfD for a single chemical, and its use in a risk characterization, e.g., a Hazard Index calculation (see Section 4.2.), judged similarly. An analogous approach can be taken to calculate an RfC (U.S. EPA, 1994) or a slope factor (U.S. EPA, 1987, 1996). Data on similar mixtures can be considered for developing risk estimates by using a comparative potency approach that requires consistency of potency estimates across several bioassays (Section 3.2).

3.1.2. Examples of RfD Development for a Whole Mixture. Among the first mixture RfDs were those developed by the Agency's Reference Dose/Reference Concentration Work Group (RfD/C WG) for the commercial PCB mixtures, Aroclor 1016, Aroclor 1248 and Aroclor 1254 in the early 1990's, with the resulting information made available on IRIS (U.S. EPA, 1998). RfDs were derived for Aroclor 1016 and Aroclor 1254, but Aroclor 1248 was deemed "not verifiable."

Some details on Aroclor 1016 are provided below to illustrate this procedure for a whole mixture. For additional information, see the IRIS Data Base.

Aroclor 1016

After a review of the spectrum of effects found in available studies on Aroclor 1016, the RfD/C WG selected a critical effect of reduced birth weights in a monkey reproductive bioassay (Barsotti and van Miller, 1984) to establish an RfD of 7E-5 mg/kg/day. This assessment was supported by a series of reports that evaluated perinatal toxicity and long-term neurobehavioral effects of Aroclor 1016 in the same groups of infant monkeys (Levin et al., 1988; Schantz et al., 1989, 1991). An uncertainty factor (UF) of 100 was used: a 3-fold factor is applied to account for sensitive individuals; a 3-fold factor for extrapolation from rhesus monkeys to humans; a 3-fold factor for limitations in the data base, particularly relative to the issue of male reproductive effects; and a 3-fold factor for extrapolation from a subchronic exposure to a chronic RfD.

The NOAEL was selected and UFs applied as if Aroclor 1016 were a single chemical. The RfD/C WG did, however, provide statements concerning the uncertainty in this assessment, its applicability to humans, and its use by risk assessors given that the substance is a mixture. The guidance that was provided on IRIS includes:

> Confidence in the critical studies is rated medium since essentially only one group of monkeys has been examined. The initial study was well conducted in a sensitive animal species (rhesus monkeys) that closely resembles humans for many biological functions. These studies evaluated many sensitive endpoints of PCB toxicity and the effects observed have also been documented for human exposure.
>
> The data base for PCBs in general is extensive. Studies examining Aroclor 1016 have been performed in rhesus monkeys, mice, rats and mink. However, despite the extensive amount of data available only medium confidence can be placed in the data base at this time. It is acknowledged that mixtures of PCBs found in the environment do not match the pattern of congeners found in Aroclor 1016, therefore the RfD is only given medium confidence. For those particular environmental applications where it is known that Aroclor 1016 is the only form of PCB contamination, use of this RfD may rate high confidence. For all other applications only medium confidence can be given.

3.1.3. Example of Cancer Assessment for a Whole Mixture. A dose-response assessment was performed for coke oven emissions with the results loaded onto IRIS in 1989 (U.S. EPA, 1998). Coke oven emissions were determined to be a human carcinogen, causing increased risk of mortality from cancer of the lung, trachea and bronchus; cancer of the kidney; cancer of the prostate; and cancer at all sites combined in coke oven workers. The inhalation unit risk, defined as the quantitative estimate in terms of incremental or excess risk per µg/cu.m air breathed, of 6.2E-4 per (µg/cu.m) was based on respiratory cancer in males exposed in an occupational setting to coke oven emissions. This assessment is uniquely different from most cancer quantitations found on IRIS because it is based on epidemiologic data on the exposure of concern and because the coke oven emissions mixture is evaluated as if it were a single chemical. The IRIS description of the quantitative assessment of the Lloyd-Redmond cohort data (Lloyd et al., 1970; Lloyd, 1971) is as follows:

> Respiratory cancer was considered the most appropriate basis for quantitation as it was the common finding among epidemiologic studies. U.S. EPA (1984) calculated an inhalation unit risk estimate based on the Lloyd-Redmond cohort data assembled by Mazumdar et al. (1975) and sorted by Land (1976). The total background U.S. death rate was used as a basis of comparison rather than the death rate for nonwhite males. A composite unit risk estimate of 6.2E-4 per (µg/cu.m) was obtained by calculating the geometric mean of the 95% upper bound estimates obtained for four latency periods (0, 5, 10 and 15 years). This value estimates the human lifetime respiratory cancer death rate due to continuous exposure to 1 µg/cu.m of the benzene-soluble organics extracted from the particulate phase of coal tar pitch volatiles from coke oven emissions.

Although coke oven emissions are known to be a complex mixture, differences in components for the various mixtures exposures were not a part of this assessment. As indicated in IRIS, the exposures consist of either direct exposure to coke oven emissions by workers or to their extracts and condensates in animal inhalation studies and skin-painting bioassays. The general composition of these emissions is assumed to be stable. The only mention of components

is made in reference to mutagenicity studies of whole extracts and condensates, where these studies were also done on individual components. These studies provided supportive evidence for carcinogenicity.

3.1.4 Procedure for a Whole Mixture Dose-Response Assessment. If a risk assessor wants to calculate an RfD, RfC, slope factor or other dose-response estimate for a whole mixture, the general process is to assume the mixture can be treated the same as a single chemical and proceed with the established methodology for generating that estimate. The difference for the mixture assessment lies in several areas: data requirements, the establishment of the stability of the mixture so that this assumption can be made with confidence, cautions relative to dose-response models, and the need for guidance on the use of the estimate given that it is based on mixtures data. The following procedural requirements must be considered:

1) *Data collection and requirements:* Human data are preferred for the assessment from either epidemiologic studies on the exposure of concern or from human clinical studies directly on the mixture of concern (e.g., clinical studies on pesticide mixtures). In its absence, a strong animal data base, such as the primate data that were used for the Araclors is needed. These data should be supported by either animal toxicity data on the commercial mixtures or on extracts from the environmental/occupational exposure, or by human or animal toxicity data on the major components of the mixture that are deemed to be responsible for the majority of its toxic effects. Assays that describe the mode of action for the mixture are also desirable. In addition, there may be other data requirements for the methodology of the toxicity value that is being estimated, and these should be met.

2) *Stability of the mixture:* The risk assessor must ascertain that the mixture in question is relatively stable. Some of the issues that need to be considered include stability of the mixture in the environment, variability of the mixture composition over time, sources of the mixture, and potential differences between mixtures tested in the laboratory and those in the environment (e.g., bioavailability and route of exposure). In determining stability, consideration should be given to any information on the environmental exposure that may cause the components to occur in markedly different concentrations or proportions; if this is the case, information should be gathered to examine any differences in environmental fate, in uptake and pharmacokinetics, or in toxicologic effects.

3) *Dose-response assessment:* The same procedures may be used as is common for single chemical dose-response assessments. The NOAEL RfD/C approach or benchmark dose methodology with the application of appropriate uncertainty factors can be used for development of one of these values (U.S. EPA, 1998). The approaches recommended in the Proposed 1996 Cancer Guidelines (U.S. EPA, 1996) may be used to develop estimates of cancer dose-response. There should be some caution, however in applying dose-response models to whole mixture data (e.g., applying a weibull model to generate a benchmark dose or using the linearized multistage model). Dose-response models that are empirical and are based on toxicity data similar to the environmental exposure of interest are more reliable than those requiring substantial extrapolation, either to a different exposure route or to a much lower dose (concentration) than was used in the original toxicity studies. The risk assessor must recognize that dose-response models used for single compounds are often based on biological mechanisms of the toxicity of single compounds, and may not be as well justified when applied to the mixture as a whole.

4) *Guidance on the usefulness of the assessment:* The risk assessor must fully characterize the nature of the data upon which the estimate has been made, noting the relevance of the animal, epidemiologic or clinical data to environmental exposures. Investigations that were made into establishing the stability of the mixture should be disclosed with uncertainties discussed. The risk assessor must also be aware of environmental fate issues that may make the mixture too unstable to be characterized by laboratory toxicity or epidemiologic data (e.g., the mixture may exist only up to a certain distance from the emissions source). Attention should be given to the persistence of the mixture in the environment as well as to the variability of the mixture composition over time or from different sources. If the components of the mixture are known to partition into different environmental compartments or to degrade or transform at different rates in the environment, then those factors must also be taken into account, or the confidence in and applicability of the risk assessment is diminished. The confidence in the assessment must be discussed along with any cautions relative to its use in risk characterizations (see example in 3.1.2 for Aroclor 1016).

3.2. COMPARATIVE POTENCY METHOD FOR EVALUATING THE TOXICITY OF SIMILAR COMPLEX MIXTURES

3.2.1. The Comparative Potency Method. One of the few procedures for similar mixtures that has been developed and applied to data on environmental mixtures is the comparative potency method. In this procedure, a set of mixtures of highly similar composition is used to estimate a scaling factor that relates toxic potency between two different assays of the same toxic endpoint. The mixture of concern can then be tested in one of the assays (perhaps a simple assay, e.g., *in vitro* mutagenicity) and the resulting potency is then adjusted by the scaling factor to estimate the human cancer potency.

Comparative potency approaches were developed as a means of estimating the toxicity of a complex mixture in its entirety. Thus far, this method has been applied to data from the testing of mixtures of emissions released upon the combustion of organics (Albert et al., 1983; Lewtas, 1985, 1988). In addition, the comparative potency procedure has only been applied to estimation of long-term cancer unit risks, using surrogate test information from short-term cancer bioassays and *in vitro* mutagenicity assays. Comparable efforts for noncancer effects are just beginning to be developed (Gandolfi et al., 1995).

The comparative potency method involves extrapolation across mixtures and across assays. It is restricted to a set of different assays that monitor the same, single type of health effect, and to different mixtures that are considered toxicologically similar. The basic assumption is that the curves of dose response for the assays are the same shape and the relationship between any two mixtures will be the same, whichever assay is used. That means, if you stretch the curve of assay 1 to get the curve of assay 2 for mixture X, then you'll stretch it by the same amount for mixture Y. You also assume the curve of assay 1 for mixture Y is the same shape as for mixture X. Similarly, if you move the curve for X by a certain amount to obtain the curve of assay 2 from

assay 1's curve, you'd do the same for mixture Y. A toxic potency is one common single numeric summary of the dose-response curve. Using a numeric summary allows multiplication and division to move from one assay or mixture to another. Thus, if mixture X is twice as potent as mixture Y in assay 1, then X is twice as potent as Y in assay 2. This constancy of potency ratios can then be used to estimate potency for one mixture in one assay by using data from other assays and on other similar mixtures.

The comparative potency approach is an example of a similar mixtures approach to risk assessment. It is assumed that the mixture of concern can be considered a member of a class of similar mixtures based on similarity of biologic activity or reasonable expectation of a type of biologic activity based on chemical composition. In order to use a comparative potency method, the risk assessor must test the consistency of dose-response for the class of mixtures in question and test the assumption of a uniform proportionality constant between assays for all mixtures in the similarity class and for the series of bioassays under consideration.

3.2.2. Theoretical Development. The major assumption in the comparative potency method is that there exists a simple linear relationship between the mixtures' potencies from each assay for all members of the group of similar mixtures. The assays themselves, however, need not provide linear dose-response relationships. Consider an application to cancer unit risk estimation. A mixture with zero potency (i.e., it is not carcinogenic) must have zero potency in each bioassay for carcinogenicity, so the linear relationship across assays must pass through the origin (0,0) of the assay1-assay2 axes and is then a simple proportionality constant. This relationship is not chosen because it is simple, but is used because the mixtures are deemed toxicologically similar, and thus can serve as surrogates for one another. These mixtures must then change in potency from one assay to another in the same fashion.

In general, this assumption can be expressed as follows. Define:

$$\{ X_i \} = \text{group of m similar mixtures, where i=1,...,m} \quad (3\text{-}1)$$

$$\{ Aj \} = \text{the group of n bioassays, where j=1,...,n} \quad (3\text{-}2)$$

Let P represent the toxic potency. Then the above proportionality assumption can be written as:

$$P_{A2}(X_i) = k * P_{A1}(X_i) , \quad \text{for any } X_i \text{ in the similarity group} \quad (3\text{-}3)$$

where k is the proportionality constant that relates the potencies across the two assays. When there are only two assays and two mixtures, this can be illustrated as in Figure 3-1, where k_{12} represents the constant proportionality between assays Al and A2, and c_{12} represents the constant difference in potency between mixtures X_1 and X_2.

When three or more assays are used to establish the necessary relationships, there will be several such proportionality constants. In general, for assays Ar and As (where r and s are different and each in the range 1,...,n), the constant is k_{sr}:

$$P_{Ar}(X_i) = k_{sr} * P_{As}(X_i) \quad (3\text{-}4)$$

3.2.2.1. Example with Two Assays — Suppose that we wish to estimate the human cancer potency for mixture X_2; thus X_2 is the mixture of concern. Although direct estimation of human cancer potency usually comes from epidemiological or occupational studies, not actual bioassays on humans, we will stay with that nomenclature for consistency with the preceding discussion. Suppose that the available information is the following:

- the group of similar mixtures contains four mixtures X_1 through X_4.
- mixture X_1 is twice as potent for human cancer (assay A2) as it is for tumors from mouse skin painting (assay A1), and the cross-assay potency ratios for mixtures X_3 and X_4 are also roughly 2.

- the only potency estimate for X_2 is from mouse skin painting studies.

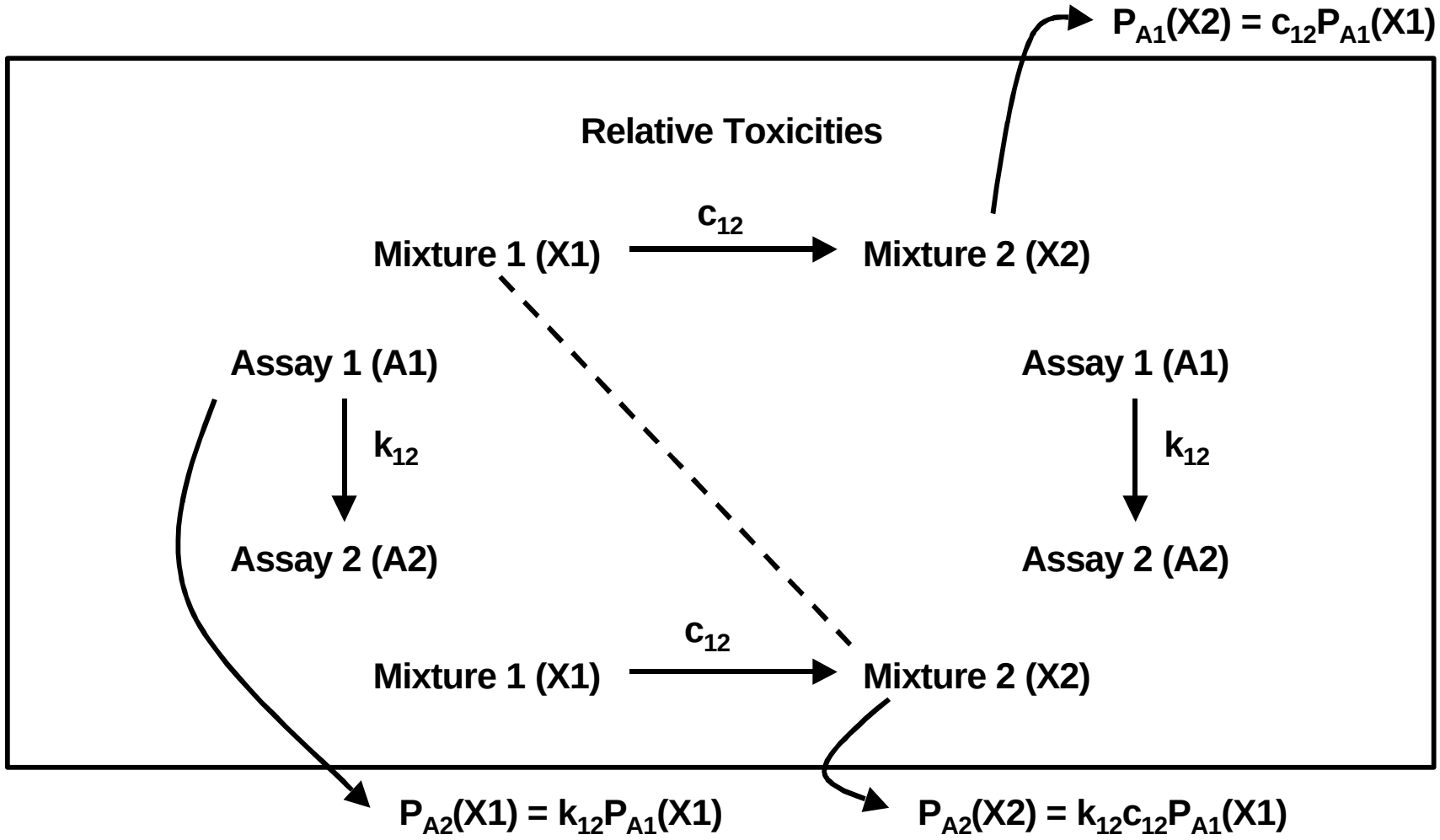


FIGURE 3-1

Proportionality Assumption for 2 Assays and 2 Mixtures

The human cancer potency for X_2 is then estimated as follows. First, k in equation 3-3 (or k_{12} in Figure 3-1) can be estimated to be 2. Because X_2 is a member of the similarity class that includes mixtures X_1, X_3 and X_4 , the same cross-assay ratio holds for X_2 as for all the other similar mixtures. From equation 3-3 and the estimate of k=2, we then have the human potency estimate for X_2 as:

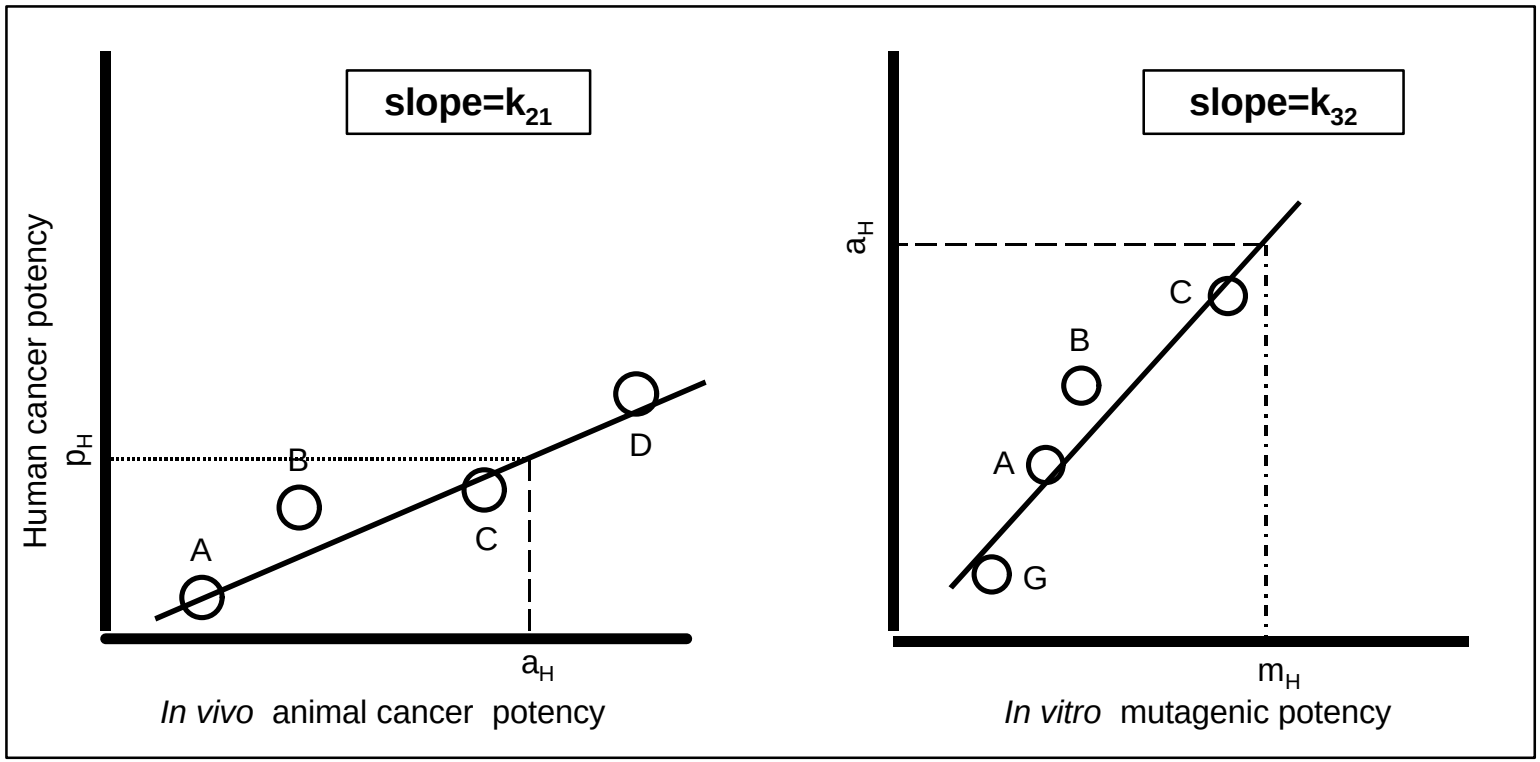
$$P_{A2}(X_2) = 2 * P_{A1}(X_2) \qquad (3\text{-}5)$$

Note that if a graph were created plotting the data for these mixtures as points with the potency for A2 on the y-axis and the potency for A1 on the x-axis, then the slope would be roughly 2. The decision to use this risk (potency) estimate from equation 3-5 is better substantiated as the graph becomes more linear.

3.2.2.2. Example with Three Assays (see Figure 3-2) — A slightly more complicated situation involves three assays, with incomplete data for each one. Suppose again that we wish to estimate the human cancer potency for mixture H, and that the available data are as follows:

- a potency estimate for mixture H has only been measured with the *in vitro* study (assay A3).
- three or more mixtures (A, B, C, G in Fig. 3-2 right) have been studied with both assays A3 and A2 (short term *in vivo* rodent study), and three or more mixtures (not the same group; A, B, C, D in Fig. 3-2 left) have been studied with both assays A2 and A1 (human cancer study).
- the two "cross-assay" constants k_{32} and k_{21} have been estimated separately using these two subsets of the class of similar mixtures.

The estimate of human potency (assay A1), using the notation in equation 3-4, is then calculated by extrapolating from assay A3 to A2 and then from assay A2 to A1. The calculation is just the potency of H from assay A3 multiplied by the product of the two cross-assay constants:



Hypothetical comparative potency example using proportionality constants with two assays.
Left) Human potency estimated from animal data for 4 mixtures.
Right) Animal potency estimated from *in vitro* data for 4 mixtures.
p_H = human potency for mixture H estimated not from the animal data but from the estimated animal potency for H, a_H, which is estimated from the *in vitro* potency, m_H., so that $p_H = k_{21} * k_{32} * m_H$.

FIGURE 3-2

Comparative Potency Method - 3 Assays

$$P_{A1}(H) = k_{32} * k_{21} * P_{A3}(H) \quad (3\text{-}6)$$

Note that, because data for H only exist with assay A3, the constants k_{32} and k_{21} are based only on data for the other mixtures (A, B, C, D, G) and do not use data on mixture H at all.

3.2.2.3. Example with Combustion Emissions — In this section, this methodology is applied to the estimation of human cancer unit risk from exposure to polycyclic organic matter (POM) from such mixtures as cigarette smoke, coke oven emissions, internal combustion engine emissions and coal burned for heat and cooking (Nesnow, 1990). The data are given in Table 3-1 and plotted in Figure 3-3. The diesel estimate for human cancer unit risk in Table 3-1 was derived based on a rat inhalation study, from a different species than the other mixtures' values. The human potency estimates for the other three mixtures are based on epidemiologic data which allows us to gauge how this potency prediction compares to the standard species-to-species extrapolation. The regression line in Figure 3-3 is based on the data without diesel, and its slope represents the cross-assay proportionality constant, or the way to scale from the mouse skin potency (A2) for diesel *via* the remaining mixtures to the human unit risk (A1) from diesel. This particular proportionality constant ($k = 4x10^{-4}$) is not significantly different from zero at one typical level of 0.05 ($p=0.14$), though the adjusted model r-square is 0.91, which suggests the model explains a lot of the variability. For our purposes, however, with only three points, a more relaxed significance level (type I error rate) (e.g., $\alpha = 0.20$) may well be good enough. So we could substitute this value of k in equation 3-3 to get:

$$P_{A1}(\text{diesel}) = (4x10^{-4}) * P_{A2}(\text{diesel}) \; . \quad (3\text{-}7)$$

TABLE 3-1

Comparative Potency Method for Emission Extracts[a]

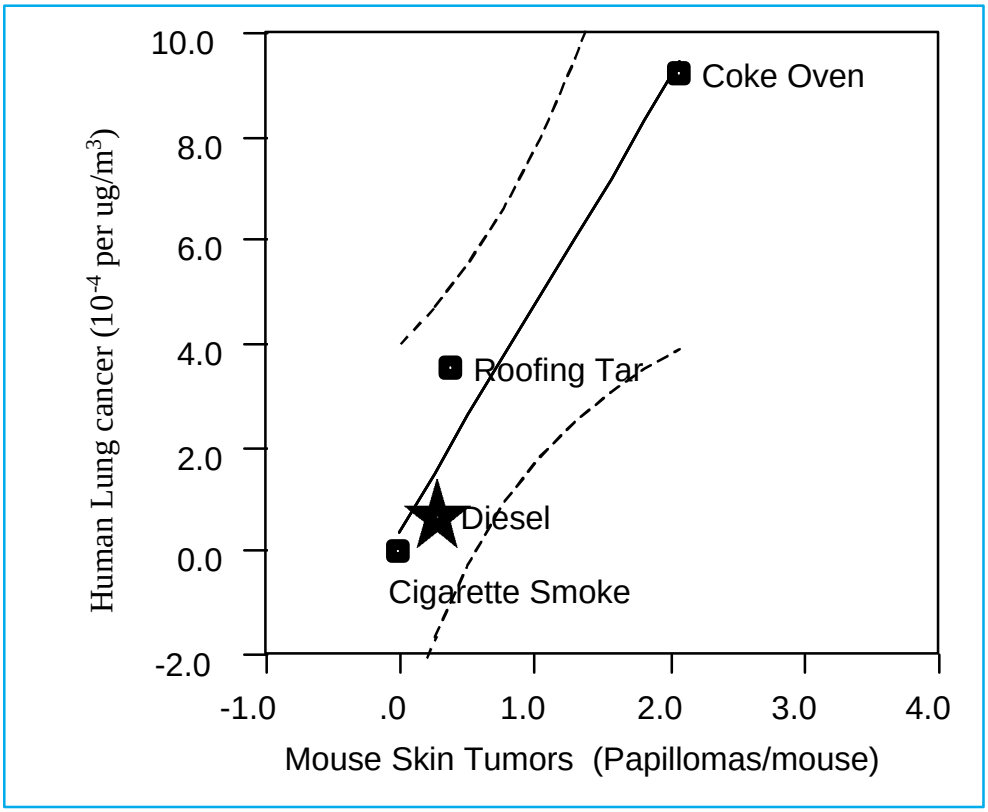
Combustion Product	Mouse Skin Tumor Initiation[b]	Human Lung Cancer Unit Risk[c] $(\mu g/m^3)^{-1}$
Coke oven emissions	2.1	$9.3x10^{-4}$
Roofing Tar	0.40	$3.6x10^{-4}$
CSC	0.0024	$2.2x10^{-6}$
Diesel	0.31	$(0.7x10^{-4})$[d]

[a] From Nesnow, 1990

[b] Expressed as number of papillomas/mouse at 1 mg organics

[c] Direct estimates from human data

[d] The diesel value was based on rat inhalation data (Albert and Chen, 1986) and was adjusted for the percentage of organics on the particulates



Linear regression shown with 95% confidence bands based on non-diesel data.

FIGURE 3-3

Combustion Mixtures (PAH)

Data from Nesnow (1990)

This estimate using comparative potency compares reasonably well with an estimate of $0.7x10^{-4}$ derived by traditional single substance methods from rodent data.

3.2.2.4. Use of Relative Potencies — Previous publications on comparative potency (Lewtas, 1985; Schoeny and Margosches, 1989) have performed the calculations using the "relative potency" (i.e., the ratio of the potency of the mixture of concern to that of a "reference mixture") in the same assay, instead of using the actual mixture potencies. Such scaling of the actual potencies does not add any information, nor does it increase the flexibility of the approach. Consider a graph of P_{A2} vs P_{A1} (i.e., the mixture potencies for assay A2 plotted against the mixture potencies for assay A1; two such graphs are shown in Figure 3-2). Scaling a quantity by a constant (e.g., the reference mixture) only changes the numbers on the axes of the graph, but the shape of the curve through the data points remains unchanged. Thus, regardless of the reference mixture used for scaling the potencies, even if different in each assay, the only relationship required is that the same proportionality constant across assays holds for all the similar mixtures.

The use of a scaled potency for comparing assays has some advantages, however, because all potencies are then "standardized" to be numbers near one (1.0), and the differences are more easily visualized. The problem occurs when tables of these standardized values are used for calculations instead of for carrying out such statistical methods as a regression. The weakness with using *relative* potencies is that the relative potency for the reference mixture (relative to itself) is always viewed as exactly 1.0; it is no longer perceived as a measured random variable but is presumed to be exact, and the variation is all assumed to lie with the other mixtures' potencies. This is clearly wrong. Consequently, regression across all mixtures should be used instead. But even when regression is used, and the index mixture value is displayed with a confidence interval (e.g., 1.0 [0.5-2.8]), the visual comparison will still tend to focus on other values in comparison

to 1.0. To avoid misinterpretation, it is better to give an analysis of the "constant ratio" assumption (i.e., the assumption of equation 3-3) separately from the table of potency data.

3.2.3. Procedure for Applying the Comparative Potency Approach. Using the comparative potency method requires gathering and analyzing data on several mixtures along with considerable judgment of toxicologic similarity. The approach should be limited to the assessment of a mixture for which whole mixture *in vivo* toxicity studies have not been done, and where the composition of the mixture is deemed too complex for the application of component-based assessment methods. Because this is a methodology based on the comparison of different mixtures and different types of data and not on an extrapolation from directly-related human health data, it is expected that these estimates will be accurate only within an order of magnitude. The following main steps have been identified:

- *Similarity of Mixtures: Develop the class characteristics or other similarity criteria for the group of mixtures, including the mixture of concern, in order to support the assumption that the group of mixtures can be judged as "toxicologically similar."*
- *Data Collection: Compile the available toxicity data on the mixtures in the similarity class and evaluate them for general quality and applicability to the toxic endpoints of interest for the mixture of concern.*
- *Potency Relationship: Estimate the degree of consistency within the mixture group across the assay ratios, and estimate values to support the constant potency ratio relationship.*
- *Dose-Response Characterization: Describe the best estimates of the cross-assay ratios along with all uncertainties in their application to human risk assessment for the mixture of concern.*

3.2.3.1. Similarity of Mixtures — The comparative potency approach is built on the assumption that the mixtures under consideration, including the mixture of concern, act in a similar manner toxicologically. A determination can be made that a group of mixtures is toxicologically similar by establishing criteria that any given mixture must satisfy in order to be designated as a member of that group. The risk assessor must be able to support the assumption

that the mixtures are similar and can do so by using any of a number of approaches that define chemical structure, biologic or statistical criteria: 1) establishing that a common mode of action exists across the mixtures; 2) showing consistency in results of short-term screening assays; 3) distinguishing chemical class or chemical structure similarity; 4) identifying common components across the mixtures in similar proportions; 5) establishing a common source of formation or emission for the group of mixtures; and 6) applying statistical criteria for similarity. Although there are references to the use of comparative potency for endpoints other than cancer (Albert, 1985), the methodology has been used by U.S. EPA only for cancer potency prediction. Use of comparative potency for non-cancer endpoints depends on the availability of accepted short-term tests relevant to those endpoints.

The mixture class characteristics that are thought most useful for prediction are those determined from data on biologic activity of the mixtures, specifically including whether the mixtures cause an effect by the same mode of action. It should be emphasized that, in estimating human potency by extrapolating from *in vivo* or *in vitro* test data, expert judgment will be needed to verify that a common mode of action may be expected to operate for the mixtures of interest across the test systems. For example, the mouse skin tumor bioassay has been shown to be an appropriate system for estimating human lung tumor potency for PAH mixtures and alkylating agents but not for metal carcinogens (Nesnow and Lewtas, 1991); the conclusion is that different *modus operandi* obtain for metals in humans than are seen in mouse lung.

Short-term screening tests can be used to determine similarity, including *in vitro* and *in vivo* models. Short-term testing to evaluate genetic toxicity (e.g., tests for DNA damage, gene mutation, cell transformation) have been suggested to characterize similar mixtures (Nesnow, 1990). Other test systems for carcinogenicity screening, such as the Syrian Hamster Embryo

(SHE) Cell Transformation Assay or the Japanese Medaka (*Oryzias latipes*), would also be candidates for short-term screening of similarity.

The identification of the major components in common for the group of mixtures can be a useful way to screen for similarity. For example, a simple chemical fractionation that indicates substantial amounts of polycyclic aromatic hydrocarbons (PAH) or aromatic amines are present, may be the basis for a preliminary grouping of similar mixtures. Nesnow (1990) suggests that common indicator constituents may be used to predict similar effects across mixtures when it can be assumed that the indicator constituents are responsible for a significant amount of the adverse effect. As the number of major components within the group of mixtures increases and the mixture becomes more complex, these methods are less reliable. EPA researchers have evaluated mixtures of up to 25 chemicals (Simmons et al., 1994), and describe difficulties in toxicologic evaluation of complex mixtures (Simmons et al., 1995). When this type of component identification is performed, care must be given to the relative proportions of the components within each of the mixtures to determine if differences in proportions are significant enough to change the type or magnitude of the effects.

An examination can be made of chemical structure activity relationships (SAR) or chemical class structure similarities in order to screen for toxicologic similarity. Nesnow (1990) suggests that genetic activity profiles can be used to identify structurally and or biologically similar chemicals (Waters et al., 1988a,b). Other SAR models can also be applied that will give indications of expected toxicity. For example, one module of the TOPKAT® structure activity relationship software that was developed for the U.S. EPA predicts the chronic rat LOAEL for chemicals by using a linear regression of the LOAEL on chemical structure descriptors (Mumtaz

et al., 1995). Other endpoints, such as the probability of carcinogenesis, can also be predicted using the TOPKAT® model (Enslein, 1990).

Consideration of the origin of the mixture provides another means for grouping; for example, mixtures resulting from incomplete combustion of organics are expected to show some degree of similarity. The degree of similarity can be pursued by combining information from origin of mixture and chemical composition of archetypal mixtures. Thus, the risk assessor could expect mixtures of POM from various types of diesel engines to constitute a similarity class; one could expect more common characteristics within this similarity sub-class than across the whole universe of combustion mixtures or with another combustion subclass (e.g., tobacco smoke condensates).

Statistical approaches can also be used to support the assumption of toxicologic similarity. One statistical evaluation of patterns of toxicologic similarity across binary interactions data has been developed for classes of chemicals (Durkin et al., 1995) using the U.S. EPA MIXTOX data base, which is a bibliographical data base of *in vivo* mammalian toxicologic interaction studies (Marnicio et al., 1991; U.S. EPA, 1990). Another statistical test, for common dose-response curves across chemical data sets, has been developed along with toxicologic criteria for deciding whether to combine cancer data sets (Vater et al., 1993; Stiteler et al., 1993). In general, the toxicologic criteria in Vater et al. (1993) are founded on considerations of data quality and on information on mode of action.

3.2.3.2. Data Collection — The act of collecting data for use in the comparative potency approach involves compiling the available toxicity data on the mixtures in the similarity class and evaluating them for general quality and applicability to the toxic endpoints of interest for the mixtures of concern. The data must be evaluated for relevance in two areas: 1) to the toxic

endpoint being assessed; and 2) for the mixture class. Assays most useful are those which can be shown to provide measures of toxicologic changes generally accepted as relevant to the mode of action. For carcinogenicity there are many short-term or limited scale assays generally considered to be relevant to processes in humans: skin-painting in rodents, *in vitro* cell transformation, development of pre-neoplastic liver cell foci, to name a few. For certain carcinogens that act by altering genetic material, it is generally accepted that mutagenicity tests *in vitro* can provide relevant data. For noncancer endpoints there are fewer well-established short-term tests, but changes in appropriate cellular receptor binding or enzyme levels are among those which could be used.

A consideration for the suitability of assay systems is similarity of pharmacokinetics among the systems and to the human situation. For most assurance of similarity, the metabolites produced and/or absorption characteristics for the chemicals/mixtures of interest should be identical (or at least comparable) across the test systems.

The data must also be evaluated in terms of providing information relevant to the human health risk assessment of the particular mixture. For example, *Salmonella typhimurium* strains widely used for *in vitro* mutation tests have an endogenous nitroreductase enzyme system not found in human cells. One would need to consider relevance of data from *Salmonella* tests when evaluating mixtures high in nitropyrenes which are easily activated by the bacteria, but may not be metabolized to carcinogens by humans.

There are numerous points in deciding whether or how to apply comparative potency. Some of these are described in Schoeny and Margosches (1989). The NRC (1988) publication *Complex Mixtures—Methods for in vivo Toxicity Testing* provides guidance not only for testing but for sampling and interpretation of data. Some decision issues are considered below.

1. *Use of extrapolation procedures.* Extrapolations that are used for the comparative potency approach should be carefully applied and justified. For example, these may include using animal data to estimate human risk, using subchronic data to estimate risk from chronic exposures, using oral or dermal data to estimate inhalation risks, or using high-dose exposures from long-term or short-term *in vitro* or *in vivo* tests to estimate risks from low exposures that humans would typically encounter in environmental media. Processes and considerations for some such extrapolations may be found in the original U.S. EPA Risk Assessment Guidelines (U.S. EPA, 1986, 1987) and subsequent guidelines for carcinogenicity, developmental toxicity, reproductive toxicity and neurotoxicity (U.S. EPA, 1996c, 1991, 1996d and 1998, respectively).

2. *Availability of human data suitable for a quantitative assessment.* The original demonstration of the comparative potency method used three combustion-related mixtures for which there were human data sufficient for derivation of a human cancer unit risk estimate (as shown in Section 3.2.2.3.). Human cancer unit risk estimates for diesel emissions from specific engine types were then derived from a central tendency estimate of the three existing human cancer unit risks on the similar combustion mixtures (Schoeny and Margosches, 1989). Greater confidence can be attached to a comparative potency approach which relies at some point on at least one human cancer unit risk estimate based on human data.

Compounds for which there are no quantitative human data could be used in the process if they are known to have a well-characterized response in an animal model that is a known reflection of human toxicity. Cancer response data from animal testing of the mixture should be evaluated following the Agency's Guidelines for Cancer Risk Assessment (U.S. EPA, 1986) and supplemented by the revised Proposed Guidelines for Cancer Risk Assessment (U.S. EPA, 1996). In using data from animals for comparative potency, care must be taken to utilize reasonable,

scientifically-based dose extrapolation processes. In particular, uncertainties introduced when extrapolating across exposure routes can be excessive and hence must be articulated and quantified when possible.

3. *Form, source and preparation of the environmental mixture sample.* Ideally the risk assessor would use data on the form of the mixture and mode of exposure most like that encountered by humans. For combustion-related mixtures, for example, the risk assessor would prefer data from inhalation assays of vapor-phase plus particulate. This type of assay is least likely to be encountered in the literature as its development is most resource intensive. The use of data from testing of the mixture in a form not presented to humans is also a source of uncertainty. For example, in the original demonstration of the comparative potency method, POM, organic extracts of combustion particulate, were tested in mouse skin initiation/promotion studies and *in vitro*. By contrast humans would be most often exposed (at least through inhalation) to a combustion mixture consisting of volatile materials and mixed sizes of particles associated with organic and inorganic compounds. The NRC (1988) gives useful guidance on collecting representative samples and their preparation for bioassay. In choosing to use data from fractions (such as organic extractables from particulate matter) or more feasible modes of administration (such as skin painting) the risk assessor introduces further areas of uncertainty into the estimate of risk. It is necessary to describe these uncertainties, limit and quantify them to the extent possible and provide justification for decisions made in data or assay choice. Point of sampling and preparation of sample must also be considered and the decisions explained. An example of a decision-making process and justification for decisions is found in Albert et al. (1983). Some considerations for data collection specific to short-term tests are found in Schoeny and Margosches (1989) and Nesnow (1991).

3.2.3.3. Potency Relationships — The next step is to estimate the degree of consistency in the assay ratios across the similar mixtures and estimate values to support the constant relative potency relationship. Having selected appropriate data types, the risk assessor then evaluates the hypothesis of consistent relative potency. If relative potency ratios are consistent across similar mixtures for one type of assay, but not others, it indicates the limitations of application of comparative potency. In other words, if only assays relating to cancer as an endpoint are consistent, the comparative potency estimation should be limited to cancer; if only receptor binding is consistent, the application should be limited to health endpoints associated with receptor binding. If there are data applicable to only one health endpoint, the methodology should not be extended to other health endpoints. In order to estimate a constant for the relative potency assay ratios for the similar mixtures, it is recommended that a linear regression model without an intercept parameter be used as illustrated in Section 3.2.2.3.

3.2.3.4. Dose-Response Characterization — This final stage of the comparative potency approach is the most important for communication and risk management decisions. Where environmental issues are significant, the risk assessment is incomplete without a characterization of the process used to determine the dose-response value. This stage includes the calculating of human potency estimates, with a full description of the uncertainty and variability of the application. The dose-response characterization should include such information as the following:

- data quality and availability,
- criteria used to determine consistency of relative potency ratios and the parallel relationship between types of assays,
- basis for the determination that the class of mixtures qualified as sufficiently similar,

- description of any extrapolations that were made, such as route-to-route or animal to human,

- full disclosure of statistical procedures that were used, any assumptions made, and significance levels used for any hypothesis testing (e.g., significant slope parameter for the linear regression),

- explanation of the level of confidence in the final human potency estimates and an estimate of the variability inherent in these numbers.

4. COMPONENT BASED METHODS FOR EVALUATING SIMPLE MIXTURES: DECREASING SEVERITY

4.1. INTRODUCTION

The mixtures methods in this chapter rely heavily on existing U.S. EPA risk assessment information on single chemical toxicity. The current dose-response assessment methodology used by the Agency for single systemic toxicants most often is directed toward the derivation of an exposure level that is not anticipated to cause significant adverse effects. Depending on the route and duration of exposure, media of concern, and the legislative mandate guiding the risk assessments, these exposure levels may be expressed in a variety of ways, such as RfDs or RfCs for lifetime oral and inhalation exposures, respectively, short-term health advisories, or other acceptable concentrations in various media. For the purpose of this discussion, the term "acceptable level" (AL) will be used to indicate any such exposure criteria or advisories derived by the Agency. Levels of exposure (E) will be understood as estimates obtained following the most current Agency exposure assessment guidance (e.g., U.S. EPA, 1992). Conceptually similar approaches for acceptability of exposure levels such as margins of exposure (MOE) have been used in risk characterization. The following descriptions of component-based mixture methods include references but assume the reader is familiar with these single chemical risk assessment concepts and practices.

4.2. HAZARD INDEX

4.2.1. Definition. The primary method for component-based risk assessment of noncancer toxicity is the Hazard Index (HI) (Teuschler and Hertzberg, 1995), which is based on dose addition (Svendsgaard and Hertzberg, 1994; also see Section 2.5). In this guidance document, dose addition is implemented as simple similar action (Finney, 1971), where the component

chemicals act as if they were dilutions or concentrations of each other. Dose additivity may not hold for all toxic effects. Further, the relative toxic potency between chemicals may differ for different types of toxicity. In practice, the HI is then usually developed for a single specific toxic effect or for toxicity to a single target organ. A mixture may then be assessed by several HIs, each representing one toxic effect or target organ.

The HI is defined as the weighted sum of the exposure measures for the mixture component chemicals. The "weight" factor according to dose addition should be the relative toxic strength, sometimes called "potency". The goal of a component-based quantitative mixture assessment is to approximate what the mixture value would be if the whole mixture could be tested. For example, an HI for liver toxicity should then approximate the concern for liver toxicity that would have been assessed using actual toxicity results from exposure to the whole mixture.

4.2.2. Information Requirements. Empirical evidence for dose addition includes parallel log-probit dose-response curves of the component chemicals, or identical dose-response curves when the doses are scaled for relative potency as well as straight line isoboles (see section 2.5 for other definitions and for more background information). Dose addition can also be demonstrated by statistical comparisons of the observed mixture response with the estimated response derived from dose addition, although this evidence may not apply to doses other than those tested. The biological basis for dose addition is the similarity of chemical components regarding toxicologic behavior, such as toxic mechanism or endpoint. When external exposure levels are used in place of internal dose, then the similarity judgment also includes physiologic disposition (uptake, metabolism, pharmacokinetics, etc.).

The HI method is specifically recommended only for groups of toxicologically similar chemicals. In practice, because of the common lack of information on mode of action and pharmacokinetics, the requirement of toxicologic similarity is usually relaxed to that of similarity of target organs (U.S. EPA, 1989a). When additional information is available on mechanism of toxicity or on other factors that could affect tissue exposure (e.g., deposition pattern in the nose), dose additivity may not be appropriate. When evidence indicates independence of action for low to moderate exposure levels, i.e., at doses near the individual chemical NOAELs, response addition should be used (see sections 2.5 and 4.2.3). Any approach not based on dose addition must be clearly described, and the evidence for applicability at low doses must be presented.

4.2.3. Alternative Formulas. The HI can be determined in several ways, depending on the available data and on the interpretation of risks that is desired. The formula must represent dose addition as a sum of exposures scaled by each chemical's relative toxicity. The only constraint is that the units of exposure and relative toxicity should cancel, so that each term and the resultant index are dimensionless. There is no commonly accepted standard measure of toxicity for exposure levels associated with low noncarcinogenic responses, in contrast to lethal levels where the LD_{50} or LD_{10} are commonly used toxicity estimates, or slope factors for carcinogenic potency. To ensure consistency with other U.S. EPA guidance on risk assessment, lethal dose data are not recommended for use in mixture risk assessment. The approach taken in the 1986 guidelines (U.S. EPA, 1986) for estimating the relative toxicity at exposures where minimal responses are expected is to use the inverse of an acceptable level (AL). The alternatives presented in this section use different toxicity-specific doses for AL.

The guidelines formula for the HI is then quite general:

$$HI = \sum_{i=1}^{n} \frac{E_i}{AL_i} \qquad (4\text{-}1)$$

where

E = exposure level,

AL = acceptable level (both E and AL are in the same units),

n = the number of chemicals in the mixture.

In practice, the HI has usually been calculated by U.S. EPA risk assessors by using the RfD or RfC as the AL (U.S. EPA, 1989). For example, for oral exposures:

$$HI = \sum_{i=1}^{n} \frac{E_i}{RfD_i} \qquad (4\text{-}2)$$

where

E_i = daily oral intake of the i^{th} chemical,

RfD_i = EPA Reference Dose for the i^{th} chemical.

Each term in equation 4-2 is called a Hazard Quotient (U.S. EPA, 1989) and represents that chemical's contribution to the toxic endpoint of concern. This equation applies to oral exposures. For the inhalation route, the exposure measure is the ambient air concentration and, instead of the RfD, the AL is the RfC (U.S. EPA, 1994).

By modifying the above formula, one can utilize other expressions for exposure and relative toxicity that may be more appropriate for different situations. For example, for an HI representing subchronic exposures, the appropriate subchronic data should be used, both for the

exposure estimate and the AL. To ensure clarity of interpretation, the scaling factors, AL, should be carefully documented and the resulting subchronic Hazard Index must be clearly identified as representing the shorter term exposure.

The use of an acceptable level in the relative toxicity scaling factor (e.g., 1/RfD) may be overly health protective in that the RfD (or RfC) is based on the critical effect, defined as the toxic effect occurring at the lowest dose. When the HI is calculated for some different, less sensitive effect, the RfD is too low, so the factor (1/RfD) will overestimate the relative toxicity and the HI will be too large. One alternative that avoids this critical effect conservatism is to use a toxicity value that is specific to the target organ of interest and is derived similarly to an RfD (or RfC). For oral exposures, this value is called the "Target organ Toxicity Dose" or TTD (Mumtaz et al., 1997). The formula for the HI would be identical to equation 4-2, with the TTD replacing the the RfD. For inhalation exposures, a similarly defined Target organ Toxicity Concentration (TTC) could be used. This same approach can be applied to HIs for shorter exposures by using the effect-specific data appropriate to the shorter exposure period of concern.

The TTD is not a commonly evaluated measure and presently there is no official U.S. EPA activity deriving these values as there is for the RfD and RfC. This alternative then should be considered when there is sufficient reason to believe that the overestimate of the HI caused by use of RfDs is significant to the interpretation of the mixture assessment. In that case, TTDs can be derived for the mixture components of interest by following the scientific steps used in deriving an RfD. The evaluation of quality of the candidate toxicity studies and the choice of uncertainty factors should parallel those steps in the RfD process. One difference in the uncertainty factors concerns the factor for completeness of the data base used for RfD development. For example, if no two-generation study existed for a chemical, there could be an additional uncertainty factor

used to obtain the RfD because the RfD must protect against all toxic effects. When developing a renal TTD, however, no additional factor would be used because the data would only include renal effects (Mumtaz et al., 1997).

Any TTDs derived for a mixture assessment must be clearly documented including the array of studies considered, the study and dose selected for calculation purposes, and the uncertainty factors chosen. When the critical effect of a chemical is the effect being described by the HI, the RfD and TTD will apply to the same target organ and so should be the same unless the TTD is based on newer information. When data for one or more components are not sufficient for deriving their organ-specific TTDs, their RfDs should be used and noted as a source of possible overestimation of the Hazard Index. This discussion and recommendations also apply to HIs for shorter exposures, and to TTCs as replacements for RfCs in an HI for inhalation exposures.

Example. Consider a mixture of six chemicals, with data given in Table 4-1. When data were not sufficient for deriving a TTD, the RfD was used as a surrogate. There were several instances, however, where the critical effect of a component is the effect of concern, so the TTD and RfD are the same. This example illustrates that, for some endpoints, the substitution of the TTD will produce an HI value that is significantly less than the HI based on RfDs alone, while for others the difference is minor. In this example, the HI for reproductive effects changes from 3 to 1 by substituting the TTDs for the RfDs, whereas the HI for renal effects only changes from 2 to 1. See Mumtaz et al. (1997) for more complete discussion of this and other examples.

These two Hazard Index methods, by using a TTD or RfD, have a quantitative weakness. The relative toxicity scaling factor (e.g., 1/RfD) is calculated from an experimental data point (e.g., the highest NOAEL). As a result, the use of small experimental dose groups could produce

no significant response (the NOAEL) solely because of the low capability to detect the effects (i.e., lack of statistical power), thereby overestimating the NOAEL and underestimating the relative toxicity. In addition, because the scaling factor is tied to actual experimental doses, wide dose spacing limits the measure's precision.

A different approach to determining relative toxicity is to calculate a benchmark dose or benchmark concentration (BMD/C) for the target organ of interest (U.S. EPA, 1996). To illustrate, consider oral exposures. The BMD approach entails identifying a dose (e.g., the ED_{10}) associated with a particular benchmark risk or magnitude of response (e.g., 10%) for the effect of concern and includes statistically fitting a dose-response model to the toxicity data. For most mixtures, however, the available dose-response data for the different component chemicals will be based on different conditions, such as differences in exposure duration or test species. The HI can use these BMDs only if some sort of standardization is applied so that the 1/BMD scaling factors describe a common scenario.

For example, if all component chemicals had chronic dose-response data on humans, then the data are already consistent and the HI would use 1/BMD for each relative toxicity scaling factor. The mixture risk could then be interpreted fairly precisely. When the HI=1, the mixture is at its BMD. For example, if the BMD is defined as the ED_{10} , then when HI=1, the mixture exposure should produce a 10% response.

When the chemical components do not have similar dose-response scenarios, some other method must be used to standardize the BMDs. An obvious approach is to use uncertainty factors and derive a TTD from each BMD, and then use 1/TTD for the scaling factor.

4.2.4. Recommended Approach. The human effect-specific BMD approach is clearly the best of the four alternative Hazard Index methods because it statistically evaluates the data

and presents a fairly precise and easily interpretable result. The TTD derived from BMDs is second best because it uses statistics, although at an intermediate step, and uses only data on the effect of concern. It is weaker because the use of uncertainty factors complicates the interpretation of the resulting HI. The straight TTD approach is third because, in addition to the UFs, it uses NOAELs or LOAELs instead of statistical evaluation of the dose-response data. The RfD approach is fourth because it not only uses NOAELs or LOAELs and uncertainty factors, but also can be based on data more sensitive than the effect of concern.

The recommended procedure is to use human BMD/Cs as toxic potency measures derived from data on the toxic endpoint of concern and for the exposure duration of interest. The relative toxicity scaling factor is then the inverse (e.g., 1/BMD for oral exposures). When human BMD/Cs are available, then HI=1 will be easily understood as representing the benchmark risk level of the specified effect. Because HI=1 is often used as a decision threshold in risk assessment, this benchmark risk should be carefully selected to represent the boundary below which the effect is deemed not to be of concern. The most recent EPA benchmark dose guidance should be used in making that selection. The default procedure, however, is to use the ALs because of their much wider availability, standardized development process including peer review, and official stature. If peer reviewed TTD/Cs become available in EPA, then they would be preferred over RfD/Cs.

The mixture components to be included in the HI calculation are any chemical components showing the effect described by the HI, regardless of the critical effect upon which the RfD/C is based. If the effect of concern is different from the RfD's or RfC's critical effect, the relative toxicity scaling factor for that chemical will be an overestimate, and the discussion of the resulting HI must include a qualifying statement that notes the potential conservatism. For shorter term exposures, the appropriate data and calculations should be used as described in the previous

sections. Other modifications, including development and use of ad hoc TTDs, are possible but should be justified in each case and should clearly describe the underlying data used in the determination.

A separate Hazard Index should be calculated for each toxic effect of concern (U.S. EPA 1986, 1989). The target organs to be addressed by the HIs should be decided for each particular mixture assessment. The assessor should compare the dose-response curves for the different toxic effects with the estimated exposure levels (and routes) to ensure that those effects most relevant to the environmental exposure are addressed. When certain toxic effects are known to occur, but at much higher exposure levels than those being assessed, then the HI for those effects may not need to be evaluated, but an explanatory note should be included in the discussion of assumptions and uncertainties for the mixture assessment.

4.2.5. Interpretation. The Hazard Index is a quantitative decision aid that requires toxicity values as well as exposure estimates; it is then part of the risk characterization. When each organ-specific HI for a mixture is less than 1 and all relevant effects have been considered in the assessment, the exposure being assessed for potential noncancer toxicity is to be considered unlikely to result in significant toxicity. When each HI is less than 1 but important information is missing or highly uncertain, then the conclusion of unlikely toxicity is weakened, and the discussion of uncertainties must be expanded appropriately. When the applicability of dose addition is also questionable, particularly if there is some evidence of synergism among some of the component chemicals, then an HI less than 1 should be viewed cautiously and consideration should be given to developing an interactions based HI (see section 4.4).

When any effect-specific HI exceeds 1, concern exists over potential toxicity. Research suggests that target organ concordance across species is generally not good and should not be

assumed (Heywood, 1981, 1983), although some effects, such as hepatic toxicity, are more consistent across species. The specific target organ or type of toxicity that is of greatest concern for humans may not be the same as that for which the highest HI is calculated from animal studies, and should not be inferred unless considerable empirical and mechanistic information exists supporting that cross-species concordance. As more HIs for different effects exceed 1, the potential for human toxicity also increases. This potential for risk is not the same as probabilistic risk; a doubling of the HI does not necessarily indicate a doubling of toxic risk. A specific numerical value of the HI, however, is usually assumed to represent the same level of concern regardless of the number of contributing chemical components or the particular toxic effect that is being tracked.

No specific action level is proposed for the HI. Because the RfDs (and by inference the TTDs) are described as having precision no better than an order of magnitude, the HI should be rounded to no more than one significant digit. Concern should increase as the number of effect-specific HIs exceeding 1 increases. The numerical magnitude of the HI must be interpreted in the context of the supporting information. For example, as a larger number of effect-specific HIs exceed 1, concern over potential toxicity should increase. Both large and small HIs should be reviewed for large uncertainties. Small HIs can be caused by incomplete characterization of the mixture composition, by missing RfDs, or by missing exposure levels for some chemicals. A large HI can be caused by a few chemicals whose RfDs (or TTDs) are based on large uncertainty factors, or because RfDs are used in place of TTDs and are based on some effect other than the one addressed by the HI. Whenever an HI is included in a risk assessment, its value must be accompanied by a description of the quality and contribution of the supporting information and of any data gaps.

4.3. EVALUATION OF INDEPENDENTLY ACTING CHEMICALS

4.3.1. Definition. For nongenotoxic chemicals (systemic toxicants) that are effectively independent in their toxic action, response addition should be used (see section 2.5 for background). The response being added is most often the percent or fraction of test animals that show toxicity. Effect addition, where the measured responses are added, will not be considered here (see section 2.5 for discussion). Following the statistical law of independent events, the formula for response addition is as given by equation 2-8:

$$r_m = 1 - (1 - r_1)*(1 - r_2)*. . . *(1 - r_n) \quad (4\text{-}3)$$

or in more compact notation:

$$r_m = 1 - \prod_{i=1}^{n}(1 - r_i) \quad (4\text{-}4)$$

The product is the probability under independence of not responding to any of the chemicals. The second form of the formula (eq. 4-4) then clearly shows that the probability of responding to the mixture is just one minus the probability of not responding.

4.3.2. Information Requirements. For exposures to nongenotoxic chemicals within an order of magnitude of their respective RfDs or RfCs, the main determinant of using response addition is whether there is sufficient evidence of either toxicologic similarity, which suggests dose addition, or toxicologic interaction. If no such evidence can be established, then independence of toxicologic action can be assumed. Often, the practical approach is to apply response addition if the target organs are different and no other evidence exists to suggest similarity or toxicologic interaction. The application of response addition requires an estimate of the expected percent

response caused by each chemical component. In order to be added, these component responses must track the same type of toxicity, at least the same target organ.

4.3.3. Interpretation. For moderate exposure levels, the full response addition formula can be applied to each type of toxicologic effect or to each target organ. The mixture assessment then can result in several response addition estimates, one per target organ. There is insufficient information for moderate exposure levels to conclude anything regarding physiological interactions between affected target organs. For high exposure estimates, additive formulas, whether response or dose, are not generally recommended because of the higher likelihood of toxicologic or physiologic interaction between component chemicals in the mixture. For low exposure levels, i.e., near the individual chemical NOAELs, toxicologically dissimilar chemicals are not expected to interact toxicologically or physiologically, and can be assumed to be functionally independent. For the special case where all the exposure levels of dissimilar components are below their respective RfDs or RfCs, the risk of systemic toxicity can be assumed to be negligible. See section 2.5.5.3 for further guidance.

4.4. WEIGHT OF EVIDENCE FOR INTERACTIONS

4.4.1. Overview of the Issue. Regulatory decisions usually involve the assessment of chemical mixtures, though often on a chemical by chemical bases. Typical exposures, in contrast, are composed of a combination of biological, chemical and physical agents that may influence each other's adverse effects. Types of interactions among mixture components that can affect toxicologic response to the whole mixture include chemical-to-chemical, toxicokinetic, and toxicodynamic interactions (see Appendix A). The impact of these constituent interactions on toxicologic response can be additive (e.g., dose additive, where chemicals act as dilutions of each other and cause toxicity by the same mode of action), less-than-additive (e.g., dietary zinc that

inhibits cadmium toxicity through toxicokinetic interactions that reduce the amount of dietary cadmium absorbed), or greater-than-additive (e.g., enhanced carcinogenicity for asbestos and tobacco smoke). Additional information and examples of data on interactions can be found in Appendix A.

Interaction effects may result from events taking place at many possible loci in the body, including the site of toxic action or during the processes of absorption, tissue distribution, metabolism, excretion or repair. Any or all of these can vary with route of administration, age, gender, health, nutritional status, etc. With the almost infinitely large number of chemical mixtures in the environment, systematic studies relevant to the toxicology of these chemical mixtures using conventional methodologies and approaches are impossible; the development of predictive and alternative toxicology methods are imperative. An evolving approach is the utilization of physiologically based pharmacokinetic/pharmacodynamic (PB-PK/PD) modeling, coupled with model-oriented toxicology experiments (Tardiff et al., 1997). Tissue dosimetry at the PK and PD levels is achievable with simple and complex, but chemically defined mixtures. Further discussions pertinent to the available PB-PK/PD modeling and the metabolic processes have been presented in Appendix A.

Evidence of toxicologic interaction should be reflected in the mixture risk assessment (U.S. EPA, 1986). Previous risk assessments of multichemical exposures by the U.S. EPA have considered the information on interactions only in a qualitative sense. For example, a Superfund site may receive more scrutiny or its remediation may proceed faster if there were several indications of potential synergism among the detected chemicals. The clean-up goals and the estimated risk, however, would not change. Consequently, most mixtures risk assessments do not include interactions information. No standard methods are yet in place in regulatory agencies to

incorporate interactions and no biologically motivated mathematical models have been developed that could serve as a default method. The method described in this section is new. Its use is encouraged so that EPA can gain experience regarding the difficulties and advantages of an interactions-based approach and then identify ways to improve the approach.

In developing an interactions-based risk assessment method, the following constraints were established:

- the method should use readily available data or at least information that can be feasibly obtained.
- the method should include several steps, each of which could be modified or replaced when more data or biological models became available.
- the method should be plausible, either supported by some empirical cases or supported by consensus among practicing mixtures toxicologists and risk assessors.

In the method described in this section, the key assumption is that interactions in a mixture can be adequately represented as departures from dose addition. The method follows an obvious approach: to begin with the dose additive Hazard Index, and then modify its calculation to reflect the interactions results, using plausible assumptions to fill in the data gaps.

4.4.2. Modified Hazard Index Using Interaction Weight-of-Evidence.

4.4.2.1. Background — Toxicologic interactions have been mostly studied with binary mixtures. One way to include interactions in a mixture assessment is to modify the noninteractive assessment by knowledge of these binary interactions; a tacit assumption is then that higher order interactions are relatively minor compared to binary interactions. Few studies quantify interaction, and even fewer quantitatively describe the dose-dependence of the interaction. Consequently, for an approach to be able to use available data, some qualitative procedure is needed for judging the impact of the potential toxicologic interactions.

The U.S. EPA previously developed a weight-of-evidence procedure that uses binary interaction data to modify the Hazard Index (Mumtaz and Durkin, 1992). This procedure reflects the strength of the available interaction studies as well as the amounts of each component in the mixture. The first step entails a review of relevant information on all of the possible binary interactions in the mixture. Among the several factors considered are the degree of understanding of the interaction, its relevance to toxicity, and the extent of extrapolation to the exposure conditions of interest (e.g., route and species conversions). The strength and consistency of this evidence is then assigned a numerical binary weight-of-evidence (BINWOE) score. The BINWOE is then scaled to reflect the relative importance of the component exposure levels. A main property of the Mumtaz and Durkin approach is that the scaled BINWOE decreases with decreasing exposure levels, reflecting a common observation that the significance of interactions in a mixture decreases as the exposure and likelihood of response decreases. This scaled BINWOE is then used to modify the dose-additive HI as follows:

$$HI_I = HI_{ADD} * UF_I^{WOE_N} \tag{4-5}$$

where HI_{ADD} is the noninteractive HI based on dose addition, UF_I is the uncertainty factor for interactions, and WOE_N is the scaled BINWOE.

The procedure outlined by Mumtaz and Durkin (1992) is a major advance in the risk assessment of chemical mixtures. The approach is quite feasible: it uses available information along with toxicological judgment and reflects many general concepts about toxicologic interactions. When tested for consistency of application (Mumtaz et al., 1995), individuals and groups tend to develop fairly similar scores, though sometimes with different rationale.

The weaknesses in the approach are few, but important. The guidance on selecting the uncertainty factor for interactions is not given, the steps in determining the BINWOE are fairly complex, and the magnitude of the interaction is not included. The relative weights applied to the various categories of information seem arbitrary in that they lack support from empirical assessments of the influence that some key experimental variables have on the interaction consistency. Further, the formula itself (equation 4-5) may be overly simple in that the interactions and additivity components are separable, i.e., the interactions information is completely represented by the multiplicative factor UF^{WOE}, which is applied to the entire additive HI.

4.4.2.2. The Modified Procedure — The recommended procedure incorporates several changes from the original developed by Mumtaz and Durkin (1992). The main difference is seen in the formula. Instead of modifying the additive index by a single composite interaction factor, each term is modified according to the influence (interaction) of the other components, and then these modified terms are summed.

Consider the example of an HI for liver. The i^{th} term in the HI, called the Hazard Quotient (HQ_i) for the i^{th} chemical (U.S. EPA, 1989a), reflects that chemical's individual contribution to hepatic toxicity. The interactions approach then considers two contributions to toxicity: the hepatic toxicity resulting from a single chemical by itself, i.e., HQ, and the influence of all the other chemicals' interactions affecting the liver. In many cases, direct measurement of changes in liver toxicity will not be available. General changes affecting internal dose, such as the bioavailability or pharmacokinetics of the chemical, can then be substituted.

The need to focus on a single chemical's toxicity is illustrated by studies showing asymmetric interactions. For example, the influence of chemical A on chemical B's toxicity may

be synergistic, while the influence of B on A's toxicity may only be dose additive. By having two separate terms in the interactions HI, these differences are incorporated.

Component exposure levels also can affect the nature and magnitude of the interaction. The high-to-low dose extrapolation is particularly problematic for mixtures. Many dramatic interactions occur at high exposure levels, e.g., the substantial synergism between tobacco smoking and radon exposure. Several publications note the expectation that most high-dose interactions will be minimal at very low doses. Examples that include the dose dependence of the interaction, however, are sparse. Feron et al. (1995) discuss some examples where interactions occur at exposures near individual minimal-observed-effect levels while only dose-addition is apparent near individual no-effect levels; they do not present a quantitative relation between interaction and dose. The influence of the relative proportions is also of concern. For example, with respect to the loss of righting reflex in mice (Gessner, 1995), the ED_{50} isobologram for the interaction between ethanol and chloral hydrate shows synergism at low ethanol levels, but concentration additivity at higher ethanol levels. One suggestion is that the interaction should become less important as one chemical begins to dominate the mixture toxicity.

4.4.2.2.1. Formula. The interactions-based Hazard Index includes two evaluations of the weight of the evidence (WOE) for interaction for each pair of component chemicals in the mixture: one WOE for the influence of chemical A on the toxicity of chemical B, and one for the reverse. This qualitative judgment is then changed into a numerical score. Some common assumptions and desirable properties could also be included:

1) The pairwise interactions capture most of the interaction effects in the mixture.

2) The interaction is highest when both chemicals in the interacting pair are at equally toxic doses (neither chemical is dominant).

3) The interaction HI must reduce to the dose additive HI as the interaction magnitudes decrease.

4) The main toxicologic effects from the mixture exposure are limited to those effects induced by the individual component chemicals.

5) The interaction magnitude is likely to decrease as mixture dose decreases.

Many formulas could be derived that reflect these ideas. The following is recommended as an interim method that is also simple. Assumptions 1 and 4 are simplifications in the data gathering stage. Assumption 2 can then be modeled by a simple symmetric function that is maximal when $HQ_i=HQ_j$. Assumption 5 has no quantitative empirical support we could find, and may be more reflective of the reduction in toxicity as dose decreases, making detection of an interaction more difficult. Consequently, assumption 5 will not be included here. Pairwise interaction studies usually show the influence of one chemical on the toxicity of the other chemical. If each HQ is used as the measure of that component chemical's toxicity, then we can modify the Hazard Index by multiplying each HQ in the formula by a function of the following quantities: the HQs of the other chemicals (to reflect the actual component exposure levels), the estimated magnitude of each pairwise interaction, and the two WOE scores. In this way, we are incorporating the interactions by modifying each HQ by the influences of all the other potentially interacting chemicals. These modified HQs are then summed to get the interaction HI for the mixture.

The WOE procedure modifies each HQ in the formula for HI. For the i^{th} chemical, the modification means multiplying HQ_i by the sum of all the pairwise interaction contributions from the remaining chemicals (thus the summation index is for all j not equal to i). This multiplier is (each term is described below):

$$\sum_{j\neq 1}^{n} f_{ij}\ M_{ij}^{B_{ij}\theta_{ij}}$$

The full modified formula for the interactions Hazard Index, HI_{INT} , is then:

$$HI_{INT} = \sum_{i=1}^{n}(HQ_i * \sum_{j\neq 1}^{n} f_{ij}\ M_{ij}^{B_{ij}\theta_{ij}}) \quad (4\text{-}6)$$

4.4.2.2.2. Weight-of-Evidence Factor (B). The binary weight-of-evidence factor B_{ij} reflects the strength of evidence that chemical j will influence the toxicity of chemical i, and that the influence will be relevant to human health risk assessment. The factor need not be the same for the influence of chemical i on the toxicity of chemical j; i.e., $B_{ij} \neq B_{ji}$. The weight-of-evidence determination begins with a classification of the available information, followed by a conversion of that classification into a numerical weight.

The modified weight-of-evidence classification is given in Table 4-2. This scheme does not focus specifically on the types of data available to support a WOE determination, but on the interpretation of the data made by an analyst or a group of analysts. In this respect, the scheme is less directive and more flexible than the BINWOE method originally developed by Mumtaz and Durkin (1992). Further, to allow for future modification of this classification, the binary nature is not mentioned, i.e., the "BINWOE" has been replaced by simply "WOE."

The scheme is based on the assessment of the direction of an interaction, the plausibility that the interaction will occur, and the potential relevance of the interaction to human health. Four levels of confidence in the assessment—Roman numerals *I* through *IV*—are described. For each category, the weight-of-evidence determination is *not* intended to consider the magnitude of

the interaction, the dose levels at which the interaction will occur, or the relative amounts of the agents in the mixture. Similarly to the original BINWOE method, these factors are considered at a *subsequent* stage of the analysis, as detailed below. The WOE scheme is then defined as:

> Weight-of-Evidence Determination—A judgment reflecting the quality of the available information that categorizes the most plausible nature of any potential influence of one compound on the toxicity of another compound, for a given exposure scenario.

As indicated in Table 4-2, the first category, *I*, is intended to reflect essentially complete confidence that the interaction will occur in humans and, therefore, the interaction is assumed relevant to human health. A classification of *I* does not necessarily imply that the interaction has been observed in humans, or even that the interaction has been demonstrated *in vivo*. While this might often be the case, it is not necessary. The classification does indicate that, *in the judgment of the analyst or group of analysts*, an interaction will occur, the direction of the interaction can be predicted with confidence, and the nature of the interaction has clear toxicologic relevance for humans.

In this context, the term *toxicologic relevance* means both that the interaction clearly affects the health of the whole animal and that the endpoint of concern for effects on human health will be affected by the interaction. For example, assume that two chemicals are under consideration, both having RfDs based on liver damage. Also assume that a study is available that demonstrates a synergistic interaction on the kidney. Depending on the nature of other supporting evidence, the information about the kidney interaction might or might not be deemed

TABLE 4-2

Modified Weight-of-Evidence Scheme*

CATEGORIES	
I.	The interaction has been shown to be relevant to human health effects and the direction of the interaction is unequivocal.
II.	The direction of the interaction has been demonstrated *in vivo* in an appropriate animal model and relevance to potential human health effects is likely.
III.	An interaction in a particular direction is plausible but the evidence supporting the interaction and its relevance to human health effects is weak.
IV.	The information: A. Insufficient to determine the direction of any potential interaction. B. Insufficient to determine whether any interaction would occur. C. Adequate as evidence that no toxicologic interaction between/among the compounds is plausible.

*See text for more detailed descriptions of each category.

relevant to the assessment of potential interactions affecting the liver. If it is deemed relevant, the kidney study could be used to support a categorization of *I*. Otherwise, a different category would apply, as discussed below. In either case, the burden is placed on the analysts to provide the rationale for the determination.

At the other extreme, the *lowest* classification level, *IV*, encompasses three very different types of assessments. The first, *IV.A*, is that an interaction may occur, but the direction of the interaction cannot be determined. This type of classification could be based on conflicting experimental results or on *mechanistic ambiguity*. For example, suppose that two studies are available on the effect of chemical *A* on chemical *B*. Both studies use essentially identical experimental designs but they yield conflicting information on the nature of the interaction. In this case, concern that an interaction could occur might be high, but the direction of the interaction could not be determined. *Mechanistic ambiguity* is a term used by Mumtaz and Durkin (1992) to describe assessments in which considering information on the biological activity of the components could lead to different interpretations. For example, if both agents are conjugated by the same compound as part of the detoxification process, competition for the conjugating compound could lead to a greater-than-additive interaction. If, however, both agents are also oxidized by the same enzyme system to more toxic intermediates prior to conjugation, saturation of the enzyme system could lead to a less-than-additive interaction. In such a case, concern for the interaction could be high, but again the direction of the interaction could not be determined.

The second category in level *IV*, *IV.B*, is simply intended for cases in which no information is available on how the compounds are likely to interact or even to indicate that any interaction is likely. This may be considered the complete opposite of Category *I* : rather than complete certainty, *IV.B* reflects the admission of complete uncertainty.

A classification of *IV.C* is almost identical to Category *I* in that there is complete certainty. In this case, however, the certainty is that no interaction will occur. This type of classification usually indicates that one of the additivity models has been demonstrated or is very likely to apply.

These three very different states of knowledge are placed within a single category because they all have the same effect on the risk assessment of a mixture. If the direction of the interaction cannot be specified—either because of conflicting information or a lack of information—or if the interaction is known to be additive, an additive model is used in the mixtures risk assessment. Explicitly identifying these three very different states of knowledge, however, is intended to highlight the need for reflecting these differences in the verbal narrative that should accompany each risk assessment.

Any number of classifications could be constructed between the complete certainty that an interaction will occur and the acceptance or demonstration of an additivity model. Only two additional categories, *II* and *III*, are defined in the recommended system. Category *II* is intended for cases in which the data strongly support the determination that an interaction will occur in a particular direction, but in which the relevance of the interaction to human health effects, while plausible, cannot be demonstrated with a high level of assurance. Category *II* then reflects the lowest extent of extrapolation, across species or target organ, but supported by some evidence of the toxicologic similarity.

The above example of two chemicals with RfDs based on liver toxicity and available data showing an interaction on renal toxicity could fit into this category if confidence were low in the relevance of the kidney interaction to effects on the liver.

Category *III* reflects more extrapolation and hence lower levels of confidence in the assessment, either in terms of relevance to *in vivo* toxic effects or of uncertainties in the direction

of the interaction. This category is intended primarily for cases in which interactions have either been demonstrated or seem plausible, but only under experimental conditions that do not correspond to the exposure scenario of concern. For example, many studies are available on interactions from sequential exposures: a group of animals is pretreated with one chemical and then dosed with a second chemical. Various control groups or different dose levels of the two agents are used to determine if pretreatment with the first chemical has any influence on the toxicity of the second chemical. These studies are usually designed to elucidate some aspect of the mechanism of action or the metabolism of the second chemical. Depending on the specific chemicals and the nature of any supporting information, the resulting data may or may not be judged sufficiently relevant for a weight-of-evidence determination. If they are used, however, a classification of *III* will often be more appropriate than a classification of *II*.

Category *III* will also encompass cases in which a toxicologic interaction has not been demonstrated, but in which mechanistic data, while not compelling, are adequate evidence that an interaction in a particular direction is more likely than an interaction in an opposite direction and more likely than no interaction at all. In other words, mechanistic ambiguity may exist but be resolvable to an extent that the case merits a score higher than *IV.A.*

The above descriptions of types of data that might fit each of the four basic categories in the modified WOE classification are not intended to be restrictive. The nature of the data chosen to support a particular classification is left to the discretion of the analyst. This relative lack of structure is the major conceptual difference between this alternative method and the original BINWOE method of Mumtaz and Durkin (1992).

The term B_{ij} is simply the quantitative weight assigned to the qualitative WOEs (Table

4-3). Positive values indicate synergism and negative values indicate antagonism. These numerical assignments are only crude weighting factors, not specific measures of interaction. As more information becomes available on toxicologic interactions, these assignments may change.

4.4.2.2.3. Exposure Factor (f). The Hazard Quotient for a chemical is multiplied by a sum of terms that reflect the other chemicals' interactions. This sum must reduce to unity (1) when dose addition is assumed, and so must be normalized in some fashion to avoid double-counting the individual Hazard Quotients. This is accomplished for each of the other components using the term fij

$$f_{ij} = \frac{HQ_j}{(HI_{add} - HQ_i)} \quad (4\text{-}7)$$

where HI_{add} is the standard hazard index based on dose additivity. This factor then scales the interaction contribution of chemical j by its importance relative to all the other chemicals interacting with chemical i. The toxicologic importance here is represented by the Hazard Quotient.

4.4.2.2.4. Interaction Magnitude (M). The term M_{ij} represents the maximum interaction effect, as defined below, that chemical j can have on the toxicity of chemical i. As with the WOE score, B, the interaction magnitude need not be symmetric; i.e., the magnitude of

interactive influence of chemical i on the toxicity of chemical j may be different than the corresponding magnitude of chemical j on the toxicity of chemical i. The direction of the effect (synergism or antagonism) is not incorporated into M_{ij}, but is contained in the WOE score, B, as described above. Thus, if chemical j increases or decreases the toxicity of chemical i by the same amount, say, to five times as toxic or one-fifth as toxic, then M_{ij} is 5.

If the application is to the determination of clean-up levels or acceptable doses, the magnitude of influence should be applicable to the low-response region of the dose-response curve. The interaction magnitude should then be estimated from the shift in the low-response-specific dose of chemical i caused by co-exposure to chemical j. One approach is to determine what the expected mixture dose would be under dose addition and compare that with the actual mixture dose that causes the same response or effect. How low the specified response should be (e.g., whether to use the LOAEL of critical effect, ED_{10}, ED_{05}) for the interaction magnitude to be relevant to clean-up levels should be determined for each case based on the nature of the available data.

If the application is to the risk characterization of existing conditions, then it may be more appropriate to estimate the magnitude by changes in the toxic effects or the percent response. Using changes in the nature of the toxic effects is not recommended because of the difficulty and lack of standard methods for quantifying that type of change. Using percent response is also troublesome. Because the percent response is bounded by 100%, care must be taken to ensure proper interpretation. For example, in a study with a high response for chemical 1 alone, say 50%, the joint response can only increase 2-fold (to 100%) because of the interaction by chemical 2. The change at a lower exposure level, say the ED_{05}, however, might be much more as its numerical limit is a 20-fold increase. Unless the expected percent response under dose additivity

is small, the interaction magnitude should be estimated from changes in iso-effective doses, not from changes in response.

Information on interaction magnitude is generally sparse to nonexistent, even for the better studied chemicals. As recently reviewed by Krishnan and Brodeur (1991, 1994), the available data on interactions involving humans compared to that involving experimental animals do not support any generalizations about the magnitude or even the direction of interactions. In addition, relatively few large-scale studies on interactions following relatively similar experimental designs have been conducted involving experimental animals. Smyth and coworkers (1969, 1970) conducted a study on the joint action of all possible pairs of 27 chemicals administered in equivolume combinations and 53 chemical pairs administered in equitoxic concentrations. The range of predicted to observed LD_{50}s was about 0.2-5. In other words, the magnitude of the deviation from additivity for the mixtures tested was about a factor of 5. More extreme interactions have been noted, for example, the interaction described by Mehendale for the effect of chlordecone on the toxicity of carbon tetrachloride.

The default interaction magnitude is set at 5 in this guidance to reflect the studies described above. When the weight-of-evidence suggests an interaction but the magnitude of the interaction cannot be quantified, this default value of 5 should be used for the interaction parameter M. Because this value does not have strong empirical support, information specific to the chemical components of concern should be used when available. Care should be taken to ensure that the measured interactions are relevant to the low exposure levels usually involved in environmental regulations, as well as to the health endpoints of concern.

4.4.2.2.5. Weighting Factor for Relative Proportions (θ). The term $\theta_{i,j}$ reflects the degree to which components i and j are present in equitoxic amounts. The definition of *equitoxic*

is based on the relative magnitudes of the Hazard Quotients. Thus, the i^{th} and j^{th} components are said to be equitoxic if $HQ_i = HQ_j$. A measure of the deviation from equitoxic amounts for the i^{th} and j^{th} components is defined simply as the ratio $\theta_{i,j}$ of the geometric mean to the arithmetic mean:

$$\theta_{ij} = \frac{(HQ_i \bullet HQ_j)^{0.5}}{[(HQ_i + HQ_j) \bullet 0.5]} \quad (4\text{-}8)$$

Note that as HQ_i approaches HQ_j, $\theta_{i,j}$ approaches unity. As the difference between HQ_i and HQ_j increases, $\theta_{i,j}$ approaches zero.

The term $\theta_{i,j}$ is incorporated into the algorithm under the assumption that, for a given total dose of two chemicals, the greatest deviation from additivity will occur when both of the components are present in equitoxic amounts. This assumption is also explicit in Finney's model of a deviation from dose additivity (e.g., Finney, 1971, Equation 11.83, p. 262).

4.4.2.2.6. Example. The properties of the interactions-based Hazard Index and some sample calculations are presented in this section, using hypothetical chemicals so that certain points can be illustrated. Consider the following scenarios where high quality information is known on the binary interactions of the mixture components. In all three cases, the weight-of-evidence categories would be I and thus the WOE scores would be 1.0.

SCENARIO 1

All binary combinations of three chemicals are known to synergize each other by a factor of 5 for the route and duration of concern, with an interaction directly relevant to human health.

SCENARIO 2

All binary combinations of three chemicals are known to be additive for the route and duration of concern, with an interaction directly relevant to human health.

SCENARIO 3

All binary combinations of three chemicals are known to antagonize each other by a factor of 5 for the route and duration of concern, with an interaction directly relevant to human health.

In Scenario 2, each Bij is equal to zero because the three chemicals are known to be additive (category IV-C in Table 4-2). As a result, M is taken to the power of zero. Thus, whatever default value is used for M, the value of M to the power of zero is unity. Also, from equation 4-7 we see that regardless of the ratios of the components in the mixture, the sum of the f_{ij}s will equal one.

$$\sum_{j \neq i}^{n} f_{ij} = \frac{\sum_{j \neq i}^{n} HQ_j}{(HI_{add} - HQ_i)} = \frac{(HI_{add} - HQ_i)}{(HI_{add} - HQ_i)} = 1$$

In other words, the Hazard Index will not change from that based on additivity. The Hazard Index modified for interactions for Scenario 2 is then:

$$HI_{INT} = \sum_{i=1}^{n} (HQ_i * \sum_{j \neq 1}^{n} f_{ij}) = \sum_{i=1}^{n} HQ_i$$

Scenarios 1 and 3 are not quite as simple. Because these scenarios are identical except for the direction of the interaction (and hence their WOE weighting factors), only Scenario 1 will be examined in detail. If each of the chemicals in the mixture is present in equitoxic amounts, then all the Hazard Quotients are equal. Equation 4-6 yields an adjusted hazard index five times greater than the hazard index based on additivity. Note that in this simple case, both $B_{i,j}$ =1 and $\theta_{i,j}$ =1. Assuming that M is set to five (the proposed scenario says each chemical is known to potentiate the other by a factor of 5), then equation 4-6 reduces to:

$$HI_{INT} = \sum_{i=1}^{n}\left(HQ_i * \sum_{j\neq 1}^{n} f_{ij} * 5 \right) = 5 * \sum_{i=1}^{n} HQ_i$$

Thus, if the Hazard Index based on additivity were 1, the Hazard Index considering interactions would be 5. The counterpart, Scenario 3, would give an interactions-based Hazard Index of 0.2.

Suppose, however, that the mixture of chemicals 1, 2, and 3 were such that the hazard quotients of each chemical were 0.98, 0.01, and 0.01, respectively. For such a mixture, it would not seem reasonable to assume as great an interaction as in the equitoxic mixture because the relative amounts of chemicals 2 and 3 are much smaller than in the equitoxic mixture. For this 98:1:1 mixture of the three chemicals, $\theta_{i,j}$ <1 for pairs involving chemical 1, resulting in a decrease in the interactions-based Hazard Index. For the effect of chemical 2 on chemical 1, using equation 4-8 gives:

$$\theta_{12} = (.98)^{.5} / (.99/2) = 0.2, \qquad f_{12} = 0.01 / (1.00\text{-}0.98) = 0.5$$

Thus, the partial adjusted hazard quotient for just the effect of chemical 2 on chemical 1 is:

$$HQ_1 * f_{12} * M^{\theta_{12}} = 0.98 * 0.5 * 5^{0.2} = 0.676$$

By symmetry, the effect of chemical 3 on chemical 1 would also be 0.676. Thus, the adjusted hazard quotient for chemical 1 would be 1.35 [=0.676+0.676], a 38% increase over HQ_1.

By applying the same hazard quotients to the other terms in equation 4-6, the adjusted hazard quotients for chemicals 2 and 3 can be determined. The adjusted hazard quotient for chemical 2 is 0.014. Because chemical 3 is present in the same relative amount as chemical 2, the adjusted hazard quotient for chemical 3 would also be 0.014. As a result, the interactions-based Hazard Index is 1.37 [1.35+0.014+0.014] for this 98:1:1 mixture of the three chemicals. Rounding to a single significant digit would yield a hazard index of 1, essentially the same as that under the assumption of additivity. Any time one chemical dominates the mixture composition by this extent, a good approximation is that the interactions-based Hazard Index will be close to the hazard quotient for that chemical.

Other cases can be similarly calculated. For example, with the same assumptions and a mixture composition of 8:1:1, a mixture having an additive Hazard Index = 1 would have an interactions-based Hazard Index of 2.77, which would round off to 3. If the interactions evidence were only in a few studies on animals, so that the WOE was level II and thus a score of 0.75, the interactions-based Hazard Index would be 2.16, which rounds to 2.

Evidence of antagonism that is not of level I quality receives a lower score than its counterpart for synergism (Table 4-3). The influence that this protective bias has on the interactions-based Hazard Index can be seen by altering Scenario 1 (equal hazard quotients, HI=1) to have interactions all of level II quality, so that antagonism yields B=0.5 whereas synergism gives B=0.75. The results are easily observed by the multiplicative (n-fold) increase or decrease in HI:

	synergism	antagonism
interactions-based Hazard Index	3.3	0.45
n-fold increase or decrease of HI	3.3	2.2

4.4.2.3. Interpretation — Algorithms are presented here for using qualitative weight-of-evidence determinations to modify a risk assessment based on information on binary interactions. These algorithms are somewhat more flexible than those proposed by Mumtaz and Durkin (1992) in that information on the magnitude of the interaction can be explicitly incorporated, and that modifications are made to each chemical's Hazard Quotient. In addition, if specific information is available, the influence of mixture composition on magnitude of interaction can also be incorporated, and the interaction can be asymmetric, i.e., the influence for chemical 1 on toxicity of chemical 2 can be different than for chemical 2 on toxicity of chemical 1.

The methods for modifying the hazard index are based on commonly discussed principles of toxicologic interactions. The algorithms, however, do not attempt to directly model toxicologic interactions. Instead, the method should be regarded as a method for modeling "concern" for toxicologic interactions, which reflects issues of magnitude as well as likelihood. In this respect, the scheme corresponds more closely with the current use of uncertainty factors in the risk assessment of single chemicals than with an attempt to biologically model interactions. When specific information is available to model the pairwise interactions as functions of component dose, such information can be used in lieu of the default procedures outlined above. As more interactions studies are completed and more interaction mechanisms are understood, these algorithms will be revised.

4.5. REFERENCE VALUE FOR A MIXTURE

4.5.1. Derivation. When only component toxicity data are available and dose or concentration addition can be assumed, knowledge of individual chemical RfDs can be used to determine the mixture RfD (Svendsgaard and Hertzberg, 1994). One example of this is human consumption of fish (Dourson and Clark, 1990). Assuming stable exposure conditions, the mixture intake is then determined by the amount of fish eaten (i.e., total mixture dose), while the relative proportions of mixture components are constant. The regulation is then a fish RfD given as the allowable intake of fish (e.g., kg of fish flesh per day).

The calculations are straightforward (Mumtaz and Hertzberg, 1993) and represent dose addition applied to the chemical components that show similar toxicity. The easiest approach is to start with the zero-interaction equation (Berenbaum, 1989), here given for a mixture of two chemicals, and using 0.001 as the fixed response for scaling the component doses:

$$1 = d_1/D_1 + d_2/D_2 \qquad (4\text{-}9)$$

where:

d_i = dose of i^{th} chemical

D_i = dose of i^{th} chemical that produce the response of 0.05

In this equation, each dose is scaled according to "doses iso-effective with the combination" and the "effect" is defined as a small response value, say, the Benchmark Dose or ED_{05} in this example. Then the D_i values are the respective ED_{05} values (doses producing a percent response of 0.05) for the two components when exposure is to one chemical at a time. If the component doses are such that equation (4-9) is true, then the mixture dose, $d_m = (d_1 + d_2)$, is at its ED_{05}, denoted here by D_m . This is seen by representing the joint exposure by fractions of total mixture dose ($d_i = f_i * D_m$):

$$1 = f_1 * D_m/D_1 + f_2 * D_m/D_2 \quad (4\text{-}10)$$

Dividing by D_m gives:

$$1/D_m = f_1/D_1 + f_2/D_2 \quad (4\text{-}11)$$

and inverting gives the mixture ED_{05}, again valid only for fixed proportions f_1 and f_2.

Example. Let the single chemical data be:

	Chemical 1	Chemical 2
ED_{05}	20	35
fraction	0.7	0.3

Then application of equation (4-11) gives the mixture ED_{05} (denoted D_m) as:

$$D_m = 1/(0.7/20 + 0.3/35) = 1/(.044) = 23$$

A similar procedure can be used to determine the Reference Dose for the mixture (RfD_m) by interpreting the iso-effective doses to be RfDs (i.e., doses producing negligible risk of adverse effects). If we invert equation (4-11) and substitute the component RfDs for the component ED_{05}s, then we obtain:

$$RfD_m = 1 / (f_1/RfD_1 + f_2/RfD_2) \quad (4\text{-}12)$$

4.5.2. Interpretation. The reference value for a mixture, such as an RfD, is reasonable only when certain conditions occur. Most critical is that the mixture composition must be fairly constant so that total mixture intake is the only important variable. If this requirement cannot be assured, then the mixture reference value should not be calculated. Another condition is that the component chemicals are similar, so that dose addition can be applied. When toxicologic similarity cannot be assured, then either another formula must be derived, or the mixture must be tested as a whole (see chapter 3). If any other formula is employed, then it must be justified. Further,

genotoxicity and other no-threshold, low dose linear toxicity must be ruled out. The other cautions regarding component-based risk characterization also apply (see section 2.5.5.3).

One of the main limitations to accuracy of this mixture reference value is the use of component reference values. While individually they have a common definition, they do not have a common data base. As noted in the discussion of the Hazard Index (section 4.2), RfDs (and RfCs) for different chemicals are derived separately, and often represent differing degrees of quality and relevance. Interpreting the composite effect of variable quality RfDs in terms of the overall quality of the mixture RfD is a difficult process. In the extreme, when one component's reference value is clearly of marginal quality as reflected by a high uncertainty factor and few studies, the assessor should discuss the uncertainty and should consider presenting two mixture reference values: one that incorporates reference values for all chemicals, and one that excludes the highly uncertain reference value.

5. COMPONENT BASED METHODS FOR EVALUATING SIMPLE MIXTURES: DECREASING RISK

5.1. RESPONSE ADDITION

5.1.1. Background. Applicability of response-addition usually refers to a situation in which independence of action holds across chemicals, similar to the statistical definition of stochastic independence (see Section 2.5.2.2.). The original U.S. EPA guidelines for mixtures risk assessment (U.S. EPA, 1986) recommend using response-addition for assessing carcinogenic risk. Those Guidelines also describe the variations on response-addition for binary mixtures when information exists on the correlation of the two tolerance distributions.

Few empirical studies have considered response addition in any depth, and few studies have modeled cancer risk from joint exposures. In an investigation of both the multistage model and the two-stage clonal expansion model for carcinogenesis, assuming an experiment using a balanced 2x2 design with 50 animals per dose group and a strong synergistic interaction, NRC (1988) concluded:

> If exposure to both agents is reduced by 2 orders of magnitude, the additivity assumption is reasonably good. (p. 193)

and

> Additivity at low doses was also demonstrated under a general class of additive background models and under the multiplicative risk model when the relative risk for each component in the mixture is small. (p. 200)

Gibb and Chen (1986) considered implications of the multistage model. They showed that at low doses, the risks from carcinogens acting on the same stage are additive while risks from carcinogens acting on different stages are multiplicative. Brown and Chu (1988) also show for the multistage model that partial lifetime exposures to two carcinogens lead to roughly additive

relative risks. For the two-stage clonal expansion model, Kodell et al. (1991) argue that "the mixture risk is roughly additive at low doses for initiators, promoters and completers. Regrettably, for most chemicals, the stage at which a compound acts is not known, nor how many stages there are, nor even whether stages exist *per se*."

In many cases, cancer is treated by the U.S. EPA as a random or stochastic phenomenon. This sole characteristic is sufficient to motivate a default assumption of response addition. The other major assumptions used by U.S. EPA, no-threshold, low-dose linearity and interspecies scaling by body allometry, are not relevant to the premise of no interaction, although they certainly play a role in estimating the magnitude of an interaction.

This discussion will be limited to exposures of individual carcinogens that are near or below the "action level." The presumption is that exposure levels that greatly exceed individual action levels will be regulated as individual chemicals. Combined exposures then become important for risk management only when individual exposures are near the individually acceptable dose range. For example, all chemical components could be barely acceptable (i.e., just below the action level) but the combined risk could become unacceptable. For priority setting, a site could have many chemicals that are slightly above the action level and the combined risk could change the relative ranking of the site.

5.1.2. Application. When component risks are small, the cross-product terms in the statistical formula for independent events become insignificant, effectively collapsing the formula to simple addition of component risks (see Section 2.5.2.2.). In the NRC (1988) examination of the two models predominantly used by EPA for low-dose extrapolation, response addition was again shown to be a good predictor of mixture risk at low doses.

Three predictions of mixture risk can be given: response addition, greater than response addition, and less than response addition. The assumption of response-addition is then a neutral science policy. The chance of a significant error from assuming response-addition is slight, according to the above arguments for low-dose, low-risk exposures. Whenever carcinogenesis is assumed to be a stochastic phenomenon with no threshold exposure level, then any initiated pool of cells has positive probability of eventually producing a tumor. A direct result is that risks to an individual from multiple chemical exposures must be combined. Response addition directly follows from the assumption of independence, and is then a neutral approach for this combined risk. Alternatives are encouraged when information suggests another approach; however, the assumptions and calculations must be described and supported in detail.

5.1.3. Use of Upper Bound Risk Estimates. The practice of assessing cancer risk for a mixture usually involves applying response addition to the lifetime excess cancer risk values available for the individual chemicals. The common values generated by the U.S. EPA are those available on the IRIS data base. Currently, the IRIS values are considered plausible upper bounds to the actual lifetime excess cancer risk of the component chemical. Concern has often been raised that applying response addition to upper bounds will lead to unreasonably high estimates of the actual mixture risk.

Chen et al. (1990) and Kodell and Chen (1994) derive mathematical expressions for the upper limit on mixture risk, but the procedures require intensive computations. Gaylor and Chen (1996) extend this discussion and derive a simple approximation to the upper limit on the mixture risk that can be more appropriate than the simple summing of component upper bounds. The numerical consequences of Kodell and Chen (1994) suggest that the error in the simple addition of component upper bounds is small compared to other uncertainties. For example, a hypothetical

example of four chemicals showed that the largest difference from the simple sum of upper bounds occurred when one chemical dominated the mixture risk. When all chemicals were roughly equal contributors to the mixture risk, the error in the overestimate was roughly a factor of two.

Cogliano (1997) approached the question of summing upper bounds of mixture components' risks in two ways: (1) whether the sum yields an improbable estimate of overall risk *[that is, is it only remotely possible for the true sum of risks to match the sum of upper bounds]*, and (2) whether the sum gives a misleading estimate *[that is, is the true sum of risks likely to be very different from the sum of upper bounds]*. Analysis of several case studies showed that as the number of mixture components increases, summing their upper bounds yields an improbable, but not misleading, estimate of the overall risk. Simple sums of upper bounds are a good approximation of the overall risk and can be adjusted downward to give a more plausible upper bound, or even a central estimate of overall risk.

5.2. WEIGHT OF EVIDENCE FOR INTERACTIONS

5.2.1. Overview of the Issue. Response addition of known carcinogens may give incorrect risk estimates for multichemical exposure when toxicologic interactions are present. These interactions can enhance or inhibit the cancer potency or the growth or progression of altered cells. Chemicals with individually weak evidence of carcinogenicity may, in combination, show strong potential to initiate tumors.

The best example of human data on carcinogen interactions can be found from epidemiologic data on mortality from lung cancer in smokers exposed to asbestos. Hammond et al. (1979) noted that, in comparison with the lung cancer death rates for non-smokers who did not have occupational exposure to asbestos, the death rate was 5.17 times higher for asbestos

workers who did not smoke, 10.85 times higher for smokers who did not work with asbestos, and 53.24 times higher for smokers who worked with asbestos. These data indicate that death rate from lung cancer is approximately 10 times higher for asbestos workers who smoke than those who do not (Mukerjee and Stara, 1981). This observation clearly indicates that the interaction between asbestos and the promoters in tobacco smoke follows a multiplicative carcinogenic response.

When interactions have been noted, the goal of risk estimation is to include carcinogenic interactions quantitatively in the mixture risk assessment. The currently available animal data base on carcinogen interactions, and in particular on promoters, is not sufficient for recommending a general multiplicative approach for their risk assessment. For example, the slope factor for a carcinogen is estimated using cancer incidence data in an animal bioassay. The data on promotion action suitable for estimating the slope factor are either incomplete or nonexistent. Most of the animal data on promoters are on the increase in the number of papilloma or on shortening of the time to tumor. Accordingly, in absence of an adequate data base, the individual cancer response of various constituents present in the mixture should be combined using response addition to estimate the response of carcinogen mixtures with promotion activities. This response additive default approach can be followed by incorporation of a correction for interaction effects if any deviation from additivity is noted. For the interim period until the adequate data base is available in the scientific literature, the approach described below for estimating carcinogenic risk of mixtures (Woo et al, 1995) is to include qualitative judgments of the interaction potential in a relative ranking of the mixture based on carcinogenic risk.

5.2.2. Methods for Evaluating Interactions for Priority Setting.

5.2.2.1. Use of Interaction Data in Hazard Indices for Carcinogens—For known or suspected human carcinogens, past practice at U.S. EPA has been to assume low dose linearity in deriving quantitative risk estimates for environmental levels of materials. This has involved the application of mathematical models to animal bioassay or human data and the derivation of a slope factor, usually the upper bound on a low-dose linear term from a multistage model. The recently proposed revisions to the Guidelines for Cancer Risk Assessment (U.S. EPA, 1996) substantially alter this procedure. Under the Proposed Guidelines, dose response assessment and hazard identification rely on consideration of the likely mode of action of the agent in question. Data of various types relating to mode of action are used to inform decisions as to the shape of dose response curves and appropriate low-dose extrapolation. In all cases a two-step approach is taken to dose response assessment. In the first step, data in the range are modeled using a biologically based model (if applicable) or curve-fitting procedure. The observed range can be extended through use of appropriate information, not limited to animal or human cancers from long-term studies. In the second step, decisions are taken as to type of low dose extrapolation. For materials for which a hypothesis of low dose linearity can apply, a straight line is drawn from a reasonable point of departure from the low end of the observed range through the origin (default approach); the slope of the line serves as the slope factor or unit risk. If it is judged that the mode of action data supports low dose nonlinearity, a margin of exposure would be calculated using the lower end of the observable range as the point of departure.

There are many opportunities for interactions among carcinogens and between carcinogens and modifiers. There have been many reported instances of antagonism, inhibition, synergism, and promotion/co-carcinogenesis. These cannot currently be incorporated

quantitatively into the cancer risk estimate for a mixture using any validated process. It is recommended that the risk assessor provide a qualitative discussion of potential for interaction among carcinogens or between carcinogens and non-carcinogens contributing to the overall carcinogenic process of the mixture.

There are several databases which provide information on interactions for chemical pairs tested in carcinogenicity or related bioassays. Information on binary mixtures of carcinogens can be found in Arcos et al. (1988), on carcinogens and inhibitors in Bagheri et al. (1988/89), and on carcinogens and promoters in Rao et al. (1989). Information from these three sources has been combined into a computerized system called the Integral Search System (ISS).

This system, described in Woo et al. (1994), can be used to evaluate the potential for interactions between members of chemical pairs to affect cancer hazard indices. This paper also describes a procedure for calculating an interaction weighting ratio or "Hazard Modification" component. An outline of this approach is presented below as an example of a published methodology which seeks to quantify the potential influence of interactions in carcinogenic mixtures. At this time, it is not offered as a recommendation of this guidance document.

Woo et al. (1994) calculate (by response addition) a value by which they describe the "inherent hazard" of the mixture, an estimate of its carcinogenic potential. They then generate all possible binary pairs of chemicals in the mixture and search the data bases for interaction "hits" or reported instances of interactions, which may either enhance (synergism, promotion/cocarcinogenesis) or reduce (antagonism, inhibition) carcinogenic potential. The authors also infer interactions for pairs not in their data bases by using a mathematical procedure based on association with chemical classes of structurally or functionally related chemicals.

Information on both inferred and reported interactions is used in the calculation of the weighting ratio (WR), which is given by the following formula:

$$WR = \frac{1 + (p.H_{Syn} + q.H_{Pro})}{1 + (r.H_{Ant} + s.H_{Inh})} \qquad (5\text{-}1)$$

where p, q, r and s are "hazard-modification effectiveness coefficients" which reflect the effectiveness of the four types of combination effects to modify the carcinogenicity of chemicals.

H_{Syn} = observed plus inferred instances of synergism between chemical pairs in the mixture

H_{Pro} = observed plus inferred instances of promotion between chemical pairs in the mixture

H_{Ant} = observed plus inferred instances of antagonism between chemical pairs in the mixture

H_{Inh} = observed plus inferred instances of inhibition between chemical pairs in the mixture.

The authors give numerical values for the "hazard-modification effectiveness coefficients" based both on their scientific judgement and on inspection of the combination effects literature encompassed in their data bases. A WR of 1 would suggest that the additivity assumption is reasonable. A high or low WR would suggest that the overall interaction tends to deviate from additivity with a predominant hazard-enhancing or hazard-reducing interaction effect, respectively.

This methodology does not have the full formality of the BINWOE approach described in Chapter 4. Furthermore, it is not applied to the common unit risk or its counterpart. It is based on a particular literature data base and may not generalize to other chemical classes.

6. METHODS FOR EVALUATING CHEMICAL CLASSES

6.1. A RELATIVE POTENCY FACTORS METHOD

6.1.1. Introduction. The toxicity (magnitude of toxic effect) of a chemical mixture is best determined by direct toxicological evaluation. When such studies are available for the mixture's component chemicals, they are generally used to develop a hazard index (see Section 4.2.). Because of the temporal and monetary constraints imposed by direct toxicological evaluation, other approaches that rely more heavily on scientific judgement have been developed to assess the special case of the toxicity of mixtures of related compounds. The use of existing data makes these approaches faster and less expensive, but they are less certain because they employ simplifying assumptions and inferred toxicity.

For the general case, evaluation of mixtures of related chemical compounds that are assumed to be toxicologically similar can sometimes be made by using Relative Potency Factors (RPFs). The approach relies on both the existence of toxicological data at least for one component of the mixture (referred to as the index compound) and scientific judgement as to the toxicity of the other individual compounds in the mixture and of the mixture as a whole. The applicability of RPFs may be limited to certain types of effects or to a specific effect due to data limitations. The toxicity of the related compounds is predicted as a constant proportion or multiple of the index chemical. This proportionality constant is based on an evaluation of the results of a smaller set of toxicological assays or analyses of the chemical structures. This constant is called the RPF and represents the relative toxicity with respect to the index compound. For example, if compound A is judged to be one-tenth as toxic as the index compound, the RPF for compound A is 0.1. If all components of the mixture are assumed to be as toxic as the index

compound, then all of RPFs would be 1.0; conversely, if all of the related compounds have minimal toxicity, all of their RPFs could be assigned a value of 0.

In the RPF approach, an equivalent exposure is the product of the measured concentration of the mixture component and the RPF. These dose equivalents are summed to obtain an estimate of exposure in terms of the index chemical. These estimates of exposure should be considered semiquantitative in nature and do not *per se* imply quantitative estimates of risk. They must be defined as to the scope of toxicological effects that are covered, and the degree of similarity in chemical structure and mechanism of action that can be inferred from the summation of the quotients below. In general for n compounds,

Mixture Concentration as Expressed as Index Compound =

$$C_l + \sum_{i=2}^{n} [RPF_i x C_i] \qquad (6\text{-}1)$$

where

C_l = concentration of the index compound in mixture
C_i = concentration of the mixture component$_i$, and
RPF_i = the proportionality constant relative to the index compound for the mixture component$_i$.

To date, three examples of the use of RPFs to estimate the toxicity of a mixture of related compounds have been used by the Agency. Each of these examples has been developed as an interim measure pending the development of more, case specific data. For each of the three approaches, the concentration of the index compound is used to estimate the toxicity of the mixture as a whole by summing the equivalent exposures of the components (i.e., by assuming dose addition). This sum is the mixture exposure expressed as an equivalent of exposure to the index compound. The mixture toxicity is then estimated by evaluating the resulting exposure as if it were only from the index compound (i.e., by comparing the equivalent exposure to the dose-response data for the index chemical). The three classes of compounds for which relative

potency approaches have been examined by the U.S. EPA are the dioxins, the polychlorinated biphenyls (PCB), and the polycyclic aromatic hydrocarbons (PAH). Because the levels of current scientific understanding of the modes of action and the toxicologic databases for these classes of compounds differ, these three attempts have not achieved the same level of scientific acceptance.

6.1.1.1. Dioxins — In March of 1989, U.S. EPA released Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-dioxins and -dibenzofurans (CDDs and CDFs) and its 1989 update (EPA/625/3-89/016). These procedures have also been discussed and adopted internationally (Mukerjee and Cleverly, 1987; NATO/CCMS, 1988). In addition to describing the regulatory need and the consensus process, the 1989 EPA document cautiously recommended comparing available toxicological data and structure activity relationship information on dioxin class members with those of 2,3,7,8-TCDD, the index compound, to estimate the significance of exposures to the other 209 compounds in this class, termed congeners. The consequence of exposure to each compound was expressed in terms of an equivalent exposure of 2,3,7,8-TCDD by multiplying the concentrations of the individual congeners by their assigned toxicity equivalence factor (TEF), a specific type of RPF. The resulting 2,3,7,8-TCDD toxicity equivalents (TEQ) were then summed to estimate the risk associated with the mixture of these compounds. The TEFs were assigned based on such data as information regarding human carcinogenicity, carcinogenic potency based on animal studies, reproductive effects data, *in vitro* test data, and structure-activity relations. One TEF was assigned to each dioxin congener; these TEFs were assumed to encompass and apply to all health endpoints and all exposure routes for this class.

A number of toxicological assumptions were associated with this approach; these included the applicability of extrapolation from short-term to long-term health effects, similarities between

interspecies metabolism, appropriateness of high-dose to low-dose extrapolations, a common mechanism of action for all members of the class, the constancy of TEF relationships for different exposure routes and health endpoints, and the concept of dose additivity (U.S. EPA, 1989b). To better capture the uncertainty in these assumptions, all TEFs were provided as order of magnitude estimates and the Agency regards the results of dioxin TEF application as interim. The specific term TEF was applied to this class because of the wide acceptance of the approach and the broad applications (i.e., across route and health endpoints) for which it was designed. Similarly, use of the term TEQ infers a greater precision than would be true for most RPFs so that this term should not be used for the general case.

After the TEFs were developed for dioxins, seven guiding criteria were developed for the TEF approach (Barnes et al., 1991; U.S. EPA, 1991). It must be noted that a key assumption for the dioxins was that a single TEF could apply to all toxic endpoints. This means that, for example, for a given congener, the same TEF would be used to assess cancer risk and to assess potential developmental effects. The criteria were:

- Demonstrated need for an interim assessment
- A well-defined group of compounds that occur in environmental samples as mixtures
- TEF based on broad set of toxicity data covering many endpoints and many congeners
- Relative congener toxicity generally consistent across many different endpoints
- Additivity of dose (i.e., dose addition)
- A presumed common mechanism for toxic endpoint of the components
- TEF are formed through a scientific consensus.

These criteria were developed for specific application to the dioxins and dioxin-like compounds. The TEF is viewed as a specific type of application of the RPF. The criteria listed by Barnes et al. reflect the specific nature of the application to the dioxins, and dioxin-like PCB as discussed below in Section 6.1.1.2.

The assignment of consensus TEF for chlorinated dibenzo-*p*-dioxin, dibenzofurans, and biphenyls has been reevaluated by a number of expert panels including a recent one organized by the World Health Organization (WHO) in 1997 (Van den Berg et al., 1998). Based on the research into the toxicity of these compounds (e.g., Ahlborg et al., 1994), which occurred after the early TEF work in the late 1980s and early 1990s, revisions were made to the TEF that reflected a consensus judgment of the expert panel. For a given scientific study to be included in this TEF reevaluation effort, this expert panel developed explicit criteria; these were the inclusion of a reference compound in the scientific study and demonstrated effects on the relevant endpoint by both the reference compound and the study compound(s) in the scientific study. The panel agreed upon a specific ranking scheme for weighting different types of scientific studies. In this weighting scheme *in vivo* toxicity data were weighted more heavily than *in vitro* data or assessments of toxicity based on structural elements of a compound (Structural Activity Relationship (SAR) data). Within the *in vivo* toxicity data, results of chronic studies were weighted most heavily followed by subchronic studies and acute studies. Toxic responses were also weighted more heavily than adaptive responses.

The WHO expert panel (Van den Berg et al., 1998) also reevaluated the soundness of the TEF approach for this group of compounds. They "...concluded that the TEF concept is still the most plausible and feasible approach for risk assessment of..." this group of compounds. Studies have been conducted that assess the toxicity of specific dioxin, furan and PCB mixtures in whole mammals (or in cultured mammalian cell lines) and compare these measures with the TEF-predicted toxicity. The TEF-predicted toxicity was found to generally agree with a range of toxicity measures (e.g., Harris et al., 1993; Schrenk et al., 1994; Harper et al., 1995; Schmitz et al., 1996; Smialowicz et al ., 1997). However, for some toxicological responses, there appears to

be evidence for non-additive interactions as well as antagonism and potentiation (e.g., Davis and Safe, 1989; Safe, 1994; Birnbaum et al., 1985).

Interestingly, the WHO expert panel (Van den Berg et al., 1998) extended the TEF approach for this group of compounds to three classes of non-mammalian chordates and developed consensus TEF for two classes of fish and birds. The expert panel also described studies in fish and birds that test the validity of the TEF approach. The results of these efforts are described as supportive of the general assumption of dose additivity, although deviations from this assumption are identified.

6.1.1.2. PCB — The Workshop Report on Toxicity Equivalency Factors for Polychlorinated Biphenyl Congeners (EPA/625/3-91/020) reported that certain groups of PCB appear to act through a common mechanism with 2,3,7,8-TCDD. On this basis TEFs (this term was again applied rather than RPF because of the specific application to this chemical subclass related to dioxins) were proposed in this report and others (e.g., Ahlborg et al., 1994) which related the toxicity of exposure to members of these PCB subclasses to that of 2,3,7,8-TCDD. The same approach to estimating TEQ was advanced for this group (U.S. EPA, 1991). TEFs were proposed only for some members of the class and the TEFs proposed were considered applicable to only the health endpoint of cancer through this common mechanism shared with the dioxins.

When assessing PCB mixtures, it is important to recognize that both dioxin-like and nondioxin-like modes of action contribute to overall PCB toxicity (Safe, 1994; McFarland and Clarke, 1989; Birnbaum and DeVito, 1997). Because relatively few of the 209 PCB congeners are dioxin-like, dioxin equivalence can explain only part of a PCB mixture's toxicity. RPFs based

on action similar to 2,3,7,8-TCDD have been developed for 13 dioxin-like PCB congeners (Ahlborg et al., 1994), but no RPFs exist for the nondioxin-like modes of action.

Because PCB cause cancer by both dioxin-like and nondioxin-like modes of action, both dioxin-like and nondioxin-like portions of a mixture must be evaluated, either jointly or separately. When environmental concentrations of the dioxin-like congeners are available, those exposure estimates can be multiplied by the corresponding RPFs and then summed to yield the equivalent 2,3,7,8-TCDD exposure level for the dioxin-like portion of the mixture. The estimated cancer risk attributable to the dioxin-like portion of the mixture is then the cancer risk for that exposure to 2,3,7,8-TCDD. For the nondioxin-like portion, the total dose of the remaining congeners (subtracting the 13 dioxin-like congeners) can be multiplied by the slope factor that would otherwise be applied to the total PCB mixture. Then the cancer risk estimates for those two portions of the mixture (dioxin-like and nondioxin-like) can be added as an estimate of the overall cancer risk posed by the mixture. U.S. EPA (1996a) provides an example of this approach.

6.1.1.3. PAH — The Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAH) (EPA/600/R-93/089) described an RPF approach for assessing the carcinogenic risks posed by exposures to non-benzo(a)pyrene (B[a]P) PAH that had been judged by the Agency as B2 substances; i.e., probable human carcinogens. The results of mouse skin carcinogenicity assays for these non-B[a]P B2 PAH were compared with those of B[a]P to estimate cancer potency. The approach assumed that the B2 PAH had the same cancer slope factor as B[a]P. The ability of these non-B[a]P B2 PAH to elicit rodent skin tumors was quantitatively compared to that of B[a]P; the results of this quantitative comparison were expressed as an “estimated order of potency”. Because this approach was limited to the cancer endpoint, based on B[a]P exposure from a single (oral) pathway (for the derivation of the slope

factor), and considered only a small subset of the PAH, EPA has described it as an estimated order of potency. This naming reflects the uncertainty EPA felt about the application of this type of approach given the current state of science of PAH. For application, the cancer slope factor for B[a]P was multiplied by the estimated order of potency to estimate cancer risk for the B2 PAH.

6.1.2. Procedures for Developing a Relative Potency Factor (RPF) Approach. TEFs for dioxins were the first example of RPFs developed and reflect the group examined to date with the broadest database and an apparent uniform mechanism of action. The criteria for developing TEFs are more rigorous than can be met by most classes of chemicals. However, they provide the background for the procedures for development of the RPF. The RPF may be less rigorous scientifically than the TEF and its application may be constrained by the available data (Table 6-1). The RPF is viewed more broadly than the TEF and can be formulated by the following procedures. Typically RPF will be developed by a body of scientists to address specific regulatory needs.

6.1.2.1. Demonstrate Need for the Use of RPF as an Interim Estimate of Exposure— The RPF approach should only be applied when dictated by a clear regulatory need. When temporal or monetary issues preclude more thorough analyses of the chemical mixture of concern, then a RPF approach may be appropriate. All parties should realize that the RPF approach is an interim method of dose-response assessment and may be more uncertain than other methods.

TABLE 6-1 Differences Between TEF and RPF	
TEF Specific Types of RPF	RPF Generalized Case
TEF apply to all heath endpoints	RPF may be limited to specific health endpoints
TEF apply to all exposure routes	RPF may be limited to specific routes
Implies greater precision due to higher quality/more abundant data and greater certainty about MOA	Implies less precision due to lower quality/fewer data and less certainty about the MOA

6.1.2.2. Initiate the Consensus Process — Scientific consensus formation should be built into the process from the beginning. Because the RPF approach relies heavily on the judgement of scientific data, the inclusion of scientists who have expertise for a given chemical class is very important. This group can assist in determining relevant and significant studies and may know of on-going research activities that could be brought to bear on the process. This group will also be needed to evaluate the final product(s) of the approach.

6.1.2.3. Define the Class of Compounds — The compounds included in the chemical class to be considered should be well-defined. They should be described in terms of the commonalities that permit them to be combined in an RPF approach. Included in the definition of the class should be whether: they are similar chemically; they produce similar toxicologic effects; the spectrum of similar toxicologic effects is one or many, and what they are; the mechanism of action underlying the observed toxicologic effects is understood. The compounds should also be known to occur as mixtures in environmental samples. Clearly, it is important to know the

compounds involved. It is also important to describe what is not known about the chemical class of interest; this includes descriptions of the limitations of current analytical techniques, purity of the individual compounds when assayed, the costs related to chemical analysis, etc. In this step it is also important to identify which compounds or groups of compounds are not being considered, the reasons for this, and the potential impact of this missing information on the mixture risk assessment. The relative abundance of a compound should also be considered: if a particular compound is relatively rare, then large uncertainties may not be a significant factor for RPF development.

6.1.2.4. Develop the RPF —

6.1.2.4.1. Select the Index Compound. All RPFs will be based on comparisons with an index compound. It is important to have a single index compound for the RPF approach. The index compound should be the member of the group that is the best studied and has the largest body of scientific data of acceptable quality. The pertinent data include exposure data for the routes of interest and health assessment data for health endpoints of interest.

For most chemical classes the index compound will be obvious. When there is more than one potential candidate for the index compound, a judgment must be made regarding which candidate has the more extensive and higher quality data base. The index compound must also have (or be expected to have) similar toxic effects to the rest of the members of the class. Toxicologic information about the alternate compounds not selected could be used to evaluate the approach to a limited degree.

6.1.2.4.2. Describe the Scientific Basis for the RPF. The scientific criteria for RPF development need to be clearly stated. If there is a known or suspected common mode of action shared by members of the class of compounds, then it should be described. Similarly, if the RPF

is based upon empirical findings, they should be described clearly. If the toxicologic assays used to develop the RPF were ranked, the justification for the ranking and the application should be described. For example, some RPF could be assigned based on evidence of deleterious health effects in humans or study animals, reproductive effects data, *in vitro* test data or structure-activity relations. Actual evidence of deleterious human effects or reproductive effects data for some compounds is usually considered more certain than inferences based on the chemical structures of compounds.

If a single RPF is judged incapable of representing all toxic effects, then this must be clearly noted. The effects that are encompassed by the approach and the scientific reasons they are included should be described. The effects not included should also be described and the reasons for the decisions described.

6.1.2.4.3. Assign RPF. A description of the approach used to determine the RPF should be included. This description should include the qualitative and quantitative interpretations of toxicologic analyses for the compounds included in the RPF. The assignment of numerical RPF values should also be explained. For example, to better capture the uncertainty in the three examples presented in Section 6.1.1., RPFs were assigned as order of magnitude estimates. Clearly, the certainty or precision of the approach should not be overstated.

Alternatively, the group developing the RPF may decide that the body of scientific data used to determine the RPF for specific members of a chemical class may be most accurately portrayed as a range. The range of a RPF could be used to convey the level of confidence in the estimate of the compound's toxicity. The developers of the range would still be required to justify the range; this includes interpretation and impact of individual toxicologic analyses.

6.1.2.5. Characterize Uncertainty—

6.1.2.5.1. Define the Health Endpoints and Exposure Routes Covered by the Approach and *not* Covered by the Approach.

In this step the risk assessor should carefully describe the scientific support for including or excluding the various endpoints and routes in the RPF approach. The risk assessor should also describe where and how scientific judgement was employed in the process.

For the widest application a data set encompassing a variety of animal species, health endpoints, and exposure routes is needed. In the best cases the risk assessor can state with some confidence whether the effect on which the RPF is based is the most sensitive. For those classes of compounds with less than complete toxicological endpoint data for all members, it may be necessary to limit the endpoints of applicability of the proposed RPF approach. When only some endpoints are represented, it is important to state what cannot be considered and why. The risk assessor should still account for other types of adverse health effects that are not included in the RPF approach. If different RPFs are developed for different toxic endpoints, and one or more effect-specific RPFs for any class member cannot be developed, this limitation must be clearly noted as a bias toward underestimating that toxicity.

6.1.2.5.2. Determine the Consistency within the Group of Compounds Considered.

If multiple health endpoints or multiple exposure routes are covered by the RPF, the issue of consistency across routes and endpoints should be addressed. "Consistency" for any approach is difficult to define. For example, a consistent approach may result in similar predicted RPF orderings across different health endpoints and *in vitro* assay results. This type of consistency may strengthen the choice of a single RPF for multiple health endpoints or exposure routes. Statistical procedures may also be used in this determination. The significance of inconsistencies

should also be indicated and reconciled if a single RPF is adopted for multiple health endpoints or routes. These may display uncertainty surrounding the mechanism of action or uncertainty about the relationships between the class members and the index compound. Uncertainty of no more than 2 orders of magnitude across endpoints and a generally consistent trend across several endpoints or exposure routes would permit the choice of single RPF for a class or subclass of compounds. This criterion can be disregarded if the RPF is limited to a single endpoint and exposure route.

6.1.2.5.3. Assess Mode of Action. It is necessary to describe the understanding of the Mode of Action (MOA) of the class of compounds for which the RPF was developed. A common MOA for the class is also the basis for the assumption of additivity. However, in some cases the class may be linked by common effect with little information concerning the underlying MOA. The risk assessor must pose the question of "to what degree do the scientific data support this assumption?".

6.1.2.5.4. Assess Additivity of Dose Assumption. The RPF approach assumes an additivity of dose. Clearly, there is a stronger basis for the RPF when dose additivity is scientifically demonstrated by dose-response assay results that examine simple mixtures of the chemical class. Studies with mixtures of compounds within a given class that examine this assumption are important. These may support the assumption and provide a higher degree of certainty for the risk assessor. If they indicate that there are synergistic or antagonistic effects that are not being considered, then the final answer based on the RPFs may be unrealistic. Interactions noted only at high exposures, however, should be viewed cautiously because they may not occur at lower environmental exposures.

6.1.2.5.5. Examine the Issues Related to Application of the RPF. In this step the authors of the approach should describe any issues related to application of the RPF. For example, there may be direct human evidence that one or more members of a class cause a health effect and the evidence supporting the toxicity of other compounds in the class may be inferred. The user should identify the percentage of the equivalent exposure that is the result of the compounds for which there is direct human data and those for which it is inferred. This will allow the risk manager to make a more informed decision. The RPF should be carefully defined as to its underlying limitations, including the notation that the value obtained is a semiquantitative indication of exposure, and might not be extended to quantitative risk assessment. People applying the RPF should also evaluate evidence for dose and route extrapolations, including the relevance of toxicological assays to human health endpoints. Of particular importance is that the RPF may not cover all risk or all endpoints, so that other toxicology information is needed. In such cases, the discussion should clearly note the limited coverage of the assessment if based only on the RPFs.

6.1.2.6. Evaluation of the RPF — At the end of the process the participants, experts and interested scientific parties should be convened to evaluate each of the RPFs as well as the application of the approach for the group of chemicals being evaluated. The group members can agree or state disagreements with decisions related to the given data set. Since the RPF development process involves data interpretation and scientific judgement, the implementation of some form of consensus decision-making at the onset of the project should make its attainment more straight forward. The fact that the RPF is considered an interim process allows all parties to gather data and reassess the process. This also provides a broader description of the limitations of this approach and this can be conveyed to the decision maker.

Ideally, the recommendation of the RPF should be by consensus. However, if the consensus process fails, some middle ground should be attempted. A majority decision can be presented for use by the Agency, with a clear statement of the arguments pertaining to the minority opinion. Alternatively, other assumptions pertaining to the toxicity of members of a chemical class may be employed (Barnes et al., 1991). For example, when the data are judged inadequate to use the above RPF procedures, a worst case approach could be adopted where all compounds in the class are assumed to be as toxic as the index chemical (this assumes the index chemical is the most toxic). Adoption of this worst-case approach is the numerical equivalent of assigning all components an RPF of 1. An opposite approach is to ignore the potential toxicity of the poorly studied chemicals when assessing the mixture's toxicity (in which case their RPFs would be the numerical equivalent of 0). Some combination of these two extremes may be the most scientifically appropriate. For example, a set of scientific criteria could be determined where some of these members of the class could be assigned an RPF of 1 and the other members could be assigned an RPF of 0.

6.1.2.7. Research Needs — The RPF is considered to be an interim method, used pending the availability of more, case specific data. The fact that the RPF approach is considered interim allows all parties to gather data and reassess the evaluation; an example of such a re-evaluation is described for dioxin TEF in Van den Berg et al. (1998). Research needs should describe and prioritize the scientific data needed to make significant scientific improvements to the assessment of a mixture of the chemical class under consideration. Prioritization of data needs should be based upon collection of those data which will best address underlying mechanisms of action causalities of the toxicologic effects observed. The prioritization should specifically identify the data needs that would permit replacement of this interim RPF approach by the usual

dose-response assessment based on direct toxicity information. Estimates of both the expenses and the time required should also be provided.

6.1.3. Risk Characterization Using RPFs.

6.1.3.1. TEF-Based Assessments — When a mixture exposure is completely described by Toxicity Equivalency Factors (TEFs), then the mixture risk is quantitatively determined as if the mixture were solely composed of the index chemical. Risk assessments for the various endpoints or target organs are performed in the same manner as for the index chemical by itself. The uncertainty characterization, however, will be different, reflecting the quality of the additivity assumption and of the supporting data used in assigning the TEFs.

6.1.3.2. RPF-Based Assessments — When all chemical class members are assigned single RPFs, the mixture risk is based solely on the equivalent exposure level for the index chemical, and is handled similarly to the TEF-based assessment described above. When multiple RPFs are determined for one or more mixture components, a separate mixture assessment should be made for each exposure route or for each major effect or target organ, as appropriate. These evaluations are similar to the separate assessments made in the usual HI procedure.

Relative Potency Factors that do not satisfy the requirements for TEFs result in less certain quantitative mixture assessments. The discussion of uncertainties and overall quality of the risk assessment must characterize the contribution of strong RPFs to the total mixture risk estimate. When most of the mixture risk is based on inferred toxicity (i.e., the index chemical is not present or its presence accounts for only a small fraction of the quantitative risk) then the assessment should be presented both with and without the risk estimated by RPFs. Confidence in this approach for a given chemical class must be characterized in the assessment in which it is

utilized. In this way an assessor's scientific judgment of this confidence will be factored into the final risk assessment.

6.1.4. Hypothetical Example of RPF Approach. The application of relative potency factors to the estimation of risk from a mixture of compounds which exert the same toxic effect by similar mode of action can be demonstrated by the following example. A group of five structurally related chemicals is used as insecticides to protect against infestations of insects on crops. This group of chemicals exhibits cholinesterase inhibition as its primary toxicologic endpoint of concern. The chemicals also exhibit a variety of other effects, but these effects are not shared uniformly across the group and appear to be due largely to other structural components of the chemicals than those conferring cholinesterase inhibitory properties. In particular, one chemical is a carcinogen, another causes kidney lesions, and three cause nonspecific hepatic hypertrophy at higher doses. Because of the commonality of the cholinesterase inhibiting effects, but lack of commonality of other effects, a relative potency factor approach is appropriate for combining risk of cholinesterase inhibition from this group of chemicals.

The first step in developing a set of relative potency factors for a group of chemicals is evaluate the data available for each and identify the dataset that appears to be the most extensive and that best describes the toxicologic propensity of the chemicals in question. In Table 6-2, the information on the five chemicals in question is summarized. From this dataset, chlorophos was selected as the index compound to which the other four will be standardized. This selection was made based upon the availability of an extensive body of data defining the nature of the effects and dose response of the compound in a number of species, and clearly relating the effects in test species to humans. The datasets for the other compounds were not as extensive or well documented. In one case, only a few, poor quality studies were available, although they provided

TABLE 6-2

Characterization of the Toxicologic Properties of Five Cholinesterase Inhibiting Chemicals

Chemical	Study NOEL (mg/kg/day)	Test Species	Duration of Critical Study	Dataset Characteristics
Alphaphos	1.0	Rat	90 days	Poor. Few poorly documented studies.
Betaphos	10.0	Rat	2 years	Good. Many well conducted and documented studies for a broad spectrum of endpoints in multiple species.
Chlorophos	0.3	Rat	2 years	Extensive. Many well conducted and documented studies for a broad spectrum of endpoints in multiple species. Human confirmation of relevance of effects.
Ethaphos	0.06	Dog	1 week	Good. Many well conducted and documented studies for a broad spectrum of endpoints.
Deltaphos	1.5	Human	24 hours	Limited. Few studies but well conducted.

an acceptable basis for calculating an RPF. The datasets for each compound must next be evaluated to determine the critical study and effect level that will be used for calculating the RPF. Often, this may be the same as the basis for the RfD.

Using chlorophos as the index compound, the RPF for each of the chemicals can be calculated. This is done by dividing the NOEL for the critical study of chlorophos by the critical study for each compound by the NOEL. The results of this calculation for the example data are presented in Table 6-3.

In the example provided, the goal of the assessment is to determine the total risk of cholinesterase inhibition due to these five compounds in foods as result of their use as insecticides on crops. Data on the concentrations of each of the chemicals in foods are available and are also presented in Table 6-3. However, the inforation is compound specific and can not be directly combined. Using the calculated RPFs, the exposures for each of the chemicals are normalized to chlorophos equivalent exposures. These exposures can then be combined and compared to a chlorophos-based regulatory endpoint such as an RfD.

A number of simplifying assumptions are evident in this example.

- The first is that the points of departure (here, NOELs) for the dose response curves of the five chemicals in question are the most significant in determining their relative behavior. This assumes that the slope and shape of each curve will not be of significance because exposures will generally be low, and the accompanying effects will occur below or near the points of departure for each chemical.

- Another issue is that the studies used in calculating the RPFs were conducted in more than one species. The example provided combines these data assuming that interspecies differences will not be of concern. This assumption should be assessed in selecting appropriate data for calculating RPFs to ensure that interspecies differences do not bias the outcome of the assessment. Where interspecies variability is marked, RPFs should be calculated using data from a single species to the extent possible.

TABLE 6-3

Relative Potency Factors and Equivalent Exposures for Five Cholinesterase Inhibiting Chemicals

Chemical	Study NOEL (mg/kg/day)	Relative Potency Factor	Exposure (mg/kg/day)	Chlorophos Equivalent Exposure (mg/kg/day)
Alphaphos	1.0	0.3	0.15	0.05
Betaphos	10.0	0.03	0.02	6E-4
Chlorophos	0.3	1	0.25	0.25
Ethaphos	0.06	5	0.05	0.3
Deltaphos	0.15	2	0.15	0.3
Total				0.85
Percentage of RPF - Predicted Toxicity Associated with the Index Compound				30%

- The duration of the studies used in the example to calculate RPFs were different, ranging from a single day to 2 years. This example assumes that the effects of concern are not cumulative in nature. Where there is evidence that effects are cumulative, studies used for calculating RPFs should be of similar duration.

6.2. GEOGRAPHIC SITE-SPECIFIC MODIFICATIONS

6.2.1. Introduction. A mixture's composition can change over time in the environment, a phenomenon sometimes loosely called "weathering." The impact of this common scenario in mixtures risk assessment is that the exposure assessment will not fully characterize the mixture in terms of its chemical components, often because of suspected changes over time in the mixture composition or because of incomplete identification of the individual chemical components. Environmental processes that can contribute to this phenomenon include partitioning, environmental transport, chemical transformation, and preferential bioaccumulation. Partitioning refers to processes by which different fractions of a mixture separate into air, water, sediment, and soil. Because of partitioning, the portion of a mixture found in drinking water can be quite different from the portion encountered through soil contact. Environmental transport can disperse different fractions of a mixture to different locations. For example, the more volatile mixture components of a mixture released into the soil would be more likely to disperse in the air, while the more water-soluble components would be more likely to flow through groundwater. The less volatile, less soluble components would be more likely to remain in place in the soil. Chemical transformation can occur as mixture components break down or otherwise react with other chemicals in the environment. Preferential bioaccumulation occurs in living organisms as different mixture components can have different rates of metabolism and elimination.

When such environmental processes cannot be directly measured or modeled, there is potential for substantial error in the risk assessment. The risk assessment can sometimes be

modified by knowledge of the process that is generating the mixture exposure, or by information on the original mixture chemicals along with the geochemical and biochemical processes operating during their transport and over time. For example, because a mixture in soil for several years loses its more volatile components, a risk assessment based only on the original mixture composition would then overestimate the long term risk if the volatile chemicals were the primary toxicants. Some adjustment based on, say, exponential decay models calibrated for the soil composition being assessed might improve the risk estimate. Conversely, a risk assessment in air based on the original mixture would underestimate the long-term risk because the most toxic volatile chemicals would be over represented in the air. When the primary change is believed to be the degree of chlorination, some adjustment of the estimated exposure or toxic potency may be possible. One example (discussed in the section 6.2.3) concerns combinations of PCBs, for which the U.S. EPA has developed specific methodology to alter the toxic potency based on site-specific environmental factors. A more common situation occurs when the total mass of the exposure cannot be fully identified in terms of individual chemical components. One example (discussed in section 6.2.4) concerns combustion emissions where a total organic carbon (TOC) analysis shows that a portion of organic emissions has not been specifically identified or quantitated. The recommended procedure is an adjustment of the emission rates of the identified chemicals.

Whenever the mixture risk assessment is based on chemical component information and the mixture composition cannot be fully identified, the uncertainty and possible bias in the resulting risk assessment must be clearly described. Attention should also be given to the persistence of the mixture in the environment as well as to the variability of the mixture composition over time or from different sources of emissions. The assessment should also discuss

methods for improving the assessment, including gathering of more data as well as employing other measurement or extrapolation techniques.

The wide diversity in mixture compositions and site characteristics precludes any recommendation for a single approach for site-specific modification of the mixture assessment. The examples in sections 6.2.3 and 6.2.4, however, can demonstrate some of the considerations that must be part of such a modification. Other modifications based on the exposure and mixture characteristics are encouraged, as long as they are clearly described and supported with plausible concepts and empirical measurements.

6.2.2. Procedures for using environmental process information to determine mixture similarity. Environmental processes can affect both exposure and toxicity of a mixture in the environment. When a mixture is altered in the environment, it is not practical to expect toxicity information to be available for each specific environmental mixture to which humans are exposed. It is more likely that there will be toxicity information for only a few standard mixtures or mixture components. If information is available on some similar standard mixtures, then a feasible approach would be to determine which standard mixtures best resemble the environmental mixture and use the toxicity information from those standard mixtures as a surrogate for the environmental mixture's toxicity. In the case of information available on mixture components, then a component based approach may be feasible.

In either case, it is important to discuss how the mixture is altered in the environment, and which source of toxicity information provides the best surrogate. It is also important to discuss what uncertainties remain even after the best surrogate information is used to estimate risks from the environmental mixture, as mixtures encountered in the environment can be markedly different from the mixtures originally released into the environment or the mixtures subjected to toxicity

testing. Partitioning and bioaccumulation, for example, can cause substantial changes in an environmental mixture. When partitioning is involved, different exposure pathways can involve exposure to different mixture fractions, for example, the mixture fraction adsorbed to soil can be different than the mixture fraction dissolved in drinking water. When bioaccumulation is involved, the mixture fraction to which humans are exposed can be more persistent than the original mixture, as the bioaccumulated mixture can contain a higher proportion of the mixture components that resist metabolism and elimination. Note that this approach makes a link between dose-response assessment and exposure assessment, as the circumstances of exposure can alter the potency of a mixture in the environment.

Different procedures should be followed depending on the degree to which most of the components in the mixture have toxicity data available for evaluation. Guidance for using environmental process information to determine mixture similarity, given certain data scenarios, are given below:

Toxicity information available on most mixture component chemicals: component based approaches

If all relevant component chemicals have toxicity information and have been measured at the time and location where population exposure is expected, then estimate the mixture toxicity by combining the component chemical toxicities. One way is to develop a Hazard Index for each toxic endpoint of interest (section 4.2). If the chemicals are sufficiently similar to form a toxicologic class, then Relative Potency Factors can be estimated (section 6.1).

Toxicity information available on only a few mixture components: bounding estimates and the use of similar mixture data

a.) If too many chemicals lack specific exposure or toxicity information but some sense of total exposure can be obtained, then a bounding approach can be used. The mixture toxicity is estimated then as a range, from the worst case (assume all components are as toxic as the most toxic component) to the least case (assume all components are as toxic as the weakest component). Consider the environmental influences to determine how the components and mixture composition will change over time and during transport to the receptor population. Determine which

chemical components will be dominant in the population exposure, and reflect that determination by a recommendation of how close to each extreme the mixture toxicity is likely to be.

b.) If the mixture can be characterized by its source, for example as a specific commercial mixture, then the mixture exposure and toxicity might be estimated by using data on an environmentally transformed similar mixture. The use of toxicity data on transformed whole mixtures is encouraged because it obviates the need for full identification and measurement of the mixture components. The decision regarding similarity must consider information and uncertainties on differences in total exposure level, in relative proportions of components, in exposure levels of key components (high toxicity and/or exposure level), and in the proportion of unknown chemical components. These differences should be judged for the transformed mixture to which the population is exposed, not for the original mixture.

c.) If a high fraction (e.g., > 30%) of chemicals in the environmental exposure cannot be identified, the assessor must judge whether the source mixture could have been altered by some components being transformed into chemicals not in the source mixture. In that case, the unidentified chemicals should be investigated further, using test methods that artificially degrade the mixture or using extrapolation methods such as QSAR on the source mixture components. If such an investigation is not feasible, then the unknown chemicals constitute a major uncertainty in the mixture assessment, which must be clearly stated.

In addition to the uncertainties described in the procedural sections for the HI, RPFs, and whole mixture testing, the risk characterization must also discuss the extent of understanding of the transport and transformation of the component chemicals from the source to the exposed population. In particular, the characterization must include the identification of the chemical components and the assumptions and errors in determining concentrations at the point of population exposure.

6.2.3. Example: PCB mixtures. EPA's approach to assessing the cancer risk from environmental PCBs (U.S. EPA, 1996a; Cogliano, 1998) illustrates both the similar-standard-mixture approach and the relative potency approach described above. There have been no cancer bioassays for PCB mixtures as encountered in the environment, but these environmental mixtures

are being assessed using both approaches. The similar-standard-mixture approach relies on cancer bioassays for a few standard PCB mixtures formerly used in commerce, while the relative potency approach is based on a large body of experimental information that elucidates mechanisms of toxicity and quantifies their potency for a small number of PCB congeners that act like dioxin.

Composition of PCB mixtures. PCBs are chemical mixtures of variable composition. Mixture components are called "congeners," with 209 different congeners possible. Although their chemical properties vary widely, different mixtures can have many common components. Table 6-4 shows the overlapping composition of some commercial mixtures in terms of congeners with 1 to 10 chlorines. PCB mixtures manufactured in the United States carried the trademark "Aroclor" followed by a four-digit number; the first two digits are "12," and the last two digits indicate the percent chlorine by weight. Aroclor 1016, with approximately 41 percent chlorine, is an exception to this scheme.

Hazard assessment and dose-response assessment for PCBs. Toxicity information is available for several Aroclors. Among the many studies that implicate PCBs as likely to cause cancer in humans, a recent study comparing four Aroclors (Brunner et al., 1996; Mayes et al., 1998) provides the best information for distinguishing the cancer potential of different mixtures. Groups of 50 male or female Sprague-Dawley rats were fed diets with different concentrations of Aroclor 1016, 1242, 1254, or 1260; there were 100 controls of each sex. Exposure began when the rats were 6 to 9 weeks old, and the animals were killed 104 weeks later. Statistically significant increased incidences of liver tumors were found in female rats for all Aroclors and in

TABLE 6-4

Typical Composition of Some Commercial PCB Mixtures

Aroclor	1016	1242	1248	1254	1260
Mono-CBs (% wt)	2	1	-	-	-
Di-CBs	19	13	1	-	-
Tri-CBs	57	45	21	1	-
Tetra-CBs	22	31	49	15	-
Penta-CBs	-	10	27	53	12
Hexa-CBs	-	-	2	26	42
Hepta-CBs	-	-	-	4	38
Octa-CBs	-	-	-	-	7
Nona-CBs	-	-	-	-	1
Deca-CBs	-	-	-	-	-
PCDFs (ppm)	ND	0.15-4.5	NR	0.8-5.6	0.8-5.6
Chlorine content (%)	41	42	48	54	60
Production, 1957-1977 (%)	13	52	7	16	11

Sources: Compiled by U.S. EPA (1996) from other sources

- = less than 1%

ND = not detected

NR = not reported

male rats for Aroclor 1260 (see table 6-5). In female rats, Aroclor 1254 appeared most potent, followed by Aroclors 1260 and 1242, with Aroclor 1016 markedly less potent. In male rats, only Aroclor 1260 caused liver tumors.

Because these Aroclors contain overlapping groups of congeners that, together, span the range of congeners most often found in environmental mixtures, EPA concluded that all environmental PCB mixtures pose a risk of cancer. The dose-response assessment, however, was able to make distinctions in the potencies of these mixtures. Using the increased incidences of liver tumors in female Sprague-Dawley rats, central-estimate and upper-bound slope factors were calculated for each of the four tested Aroclors (see table 6-6).

Exposure assessment and risk characterization for PCBs. In the environment, PCBs occur as mixtures whose compositions differ from the Aroclors. This is because after release into the environment, mixture composition changes over time, through partitioning, chemical transformation, and preferential bioaccumulation. Partitioning refers to processes by which different fractions of a mixture separate into air, water, sediment, and soil. Chemical transformation can occur through biodegradation of PCB mixtures in the environment. Preferential bioaccumulation occurs in living organisms, which tend to concentrate congeners of higher chlorine content, producing residues that are considerably different from the original Aroclors. Thus, an Aroclor tested in the laboratory is not necessarily the best surrogate for assessing that Aroclor as altered in the environment.

EPA encourages risk assessors to consider how environmental processes alter PCB mixture composition and toxicity. Through partitioning, different portions of a PCB mixture are encountered through each exposure pathway. The mixture fraction that adsorbs to sediment or soil tends to be higher in chlorine content and persistence than the original mixture; it tends also

TABLE 6-5

Liver Tumor[a] Incidences for Aroclor Mixtures

Mixture	Dose	Females	Males
Aroclor 1260	Control[b]	1/85 (1%)**	7/98 (7%)**
	25 ppm	10/49 (20%)	3/50 (6%)
	50 ppm	11/45 (24%)	6/49 (12%)
	100 ppm	24/50 (48%)	10/49 (20%)
Aroclor 1254	Control[b]	1/85 (1%)**	7/98 (7%)
	25 ppm	19/45 (42%)	4/48 (8%)
	50 ppm	28/49 (57%)	4/49 (8%)
	100 ppm	28/49 (57%)	6/47 (13%)
Aroclor 1242	Control[b]	1/85 (1%)**	7/98 (7%)
	50 ppm	11/49 (24%)	1/50 (2%)
	100 ppm	15/45 (33%)	4/46 (9%)
Aroclor 1016	Control[b]	1/85 (1%)**	7/98 (7%)
	50 ppm	1/48 (2%)	2/48 (4%)
	100 ppm	6/45 (13%)	2/50 (4%)
	200 ppm	5/50 (10%)	4/49 (8%)

** Statistically significant ($p<0.05$) by Cochran-Armitage trend test.

[a] Hepatocellular adenomas, carcinomas, cholangiomas, or cholangiocarcinomas in rats alive when the first tumor was observed.

[b] One control group supported all experiments.

Source: Brunner (1996), reported by U.S. EPA (1996).

TABLE 6-6

Human Slope Estimates (per mg/kg-day) for Aroclor Mixtures

Mixture Study	Central Slope	Upper-bound Slope
1016, Brunner	0.04	0.07
1242, Brunner	0.3	0.4
1254, Brunner	1.2	1.5
1260, Brunner	0.4	0.5
1260, Norback	1.6	2.2

Source: U.S. EPA (1996)

to be less inclined to metabolism and elimination and, thus, higher in persistence and toxicity. Consequently, ingesting contaminated sediment or soil or inhaling contaminated dust can pose relatively high risks. On the other hand, the mixture fraction that dissolves in water or evaporates into air tends to be lower in chlorine content and persistence, so risks from ingesting water-soluble congeners or inhaling evaporated congeners would tend to be lower, in the absence of contaminated sediment or dust. Preferential bioaccumulation can have even more pronounced effects, as each species in the food chain retains persistent congeners that prove resistant to metabolism and elimination. Bioaccumulated PCBs appear to be more toxic than Aroclors and more persistent in the body. The Aroclors tested in laboratory animals were not subject to prior selective retention of persistent congeners through the food chain. For exposure through the food chain, therefore, risks can be higher than those estimated in an assessment. [This last statement is an example of characterizing uncertainties that remain even after the best surrogate information is used to estimate risks from an environmental mixture.]

To reflect these environmental processes, EPA developed a tiered approach that considers how partitioning and bioaccumulation affect each exposure pathway or situation. Three tiers are provided:

> ***High risk and persistence*** (upper-bound slope, 2 per mg/kg-d; central-estimate slope, 1 per mg/kg-d). The highest slope from table 6-6 is used for pathways where environmental processes tend to increase risk: food chain exposure, sediment or soil ingestion, dust or aerosol inhalation, exposure to dioxin-like, tumor-promoting, or persistent congeners, and early-life exposure (all pathways and mixtures).
>
> ***Low risk and persistence*** (upper-bound slope, 0.4 per mg/kg-d; central-estimate slope, 0.3 per mg/kg-d). A lower slope is appropriate for pathways where environmental processes tend to decrease risk: ingestion of water-soluble congeners and inhalation of evaporated congeners. Dermal exposure is also included, because PCBs are incompletely absorbed through the skin; however, if an internal dose has been calculated by applying an absorption factor to reduce the external dose, then the highest slope would be used with the internal dose estimate.

Lowest risk and persistence (upper-bound slope, 0.07 per mg/kg-d; central-estimate slope, 0.04 per mg/kg-d). The lowest slope from table 6-6 is used when congener or homologue analyses verify that congeners with more than four chlorines comprise less than one-half percent of total PCBs. Such a mixture composition is used to established sufficient similarity to the tested mixture Aroclor 1016.

Relative potency approach for PCBs. The World Health Organization has developed toxic equivalency factors for 13 dioxin-like PCB congeners. When dioxin-like congener concentrations are reported for an environmental sample, the mixture-based approach can be supplemented by an analysis of the dioxin toxic equivalents contributed by the dioxin-like PCB congeners. Such an analysis is particularly important when environmental processes have increased the concentrations of dioxin-like congeners as a fraction of the total PCB mixture.

Because PCBs can cause cancer through both dioxin-like and nondioxin-like mechanisms, it is important to consider the contribution from both dioxin-like and nondioxin-like mechanisms to the total risk. Risks for the dioxin-like and nondioxin-like portions of the mixture are calculated separately. For the dioxin-like portion, a relative potency approach is used. The dose of each dioxin-like congener is multiplied by its toxic equivalency factor, then these products are summed to obtain the total dioxin toxic equivalents present in the PCB mixture. This, in turn, is multiplied by the dioxin slope factor to estimate the risk from dioxin-like mechanisms. For the nondioxin-like portion, a similar-standard-mixture approach is used. The total dose of PCBs, less the dose comprising the 13 dioxin-like congeners already considered, is multiplied by the appropriate PCB slope factor as determined in the previous section. U.S. EPA (1996) provides a detailed example of these calculations.

On estimating a mixture's persistence. The persistence of PCB mixtures is sometimes characterized by a measure of half-life. EPA's assessment cautions that ascribing a half-life to a mixture is problematic if half-lives of its components differ widely. More specifically, half-life

estimates for a mixture will underestimate its long-term persistence. To illustrate, consider a mixture of two components in equal parts: one component has a half-life of 1 year; the other, 100 years. If the mixture concentration is sampled after 10 years, the half-life of the total mixture will appear to be approximately 10 years: virtually all the first component will be gone, and virtually none of the second, so about half the original mixture will remain. This half-life, however, overestimates the slow rate of decrease in the more persistent mixture fraction that remains.

6.2.4. Accounting for Unidentified Chemical Components of the Mixture. Whenever direct testing of the complete mixture is not feasible, a component-based assessment is usually employed. Such an assessment requires the identification of the component chemicals. Often, such assessments rely on lists of chemicals of primary concern, or lists based on prior measurements for similar sites or situations. Component identification is commonly incomplete. When measurements indicate that a substantial portion of the combined exposure is unidentified, some characterization of the missing components must be attempted. For example, the U.S. EPA has developed guidance for addressing the unidentified compounds in emissions from municipal combustors, such as boilers, industrial furnaces, and incinerators (U.S. EPA, 1994). The procedures have been developed specifically for RCRA combustion units, but could be considered for application to other situations involving unidentified mixture component chemicals, such as pesticides in soil after years of degradation.

Combustor emissions primarily include organics and metals. The risks from heavy metals are believed to be adequately addressed, but the risks from unidentified organic compounds could be potentially significant. The RCRA guidance (U.S. EPA, 1994) presents two approaches to estimate the toxicity from the unidentified organics.

6.2.4.1. Method 1: Assume Similar Toxicity in the Unidentified Portion — The principal assumption is that the unidentified organic chemicals are similar in toxicity and chemical properties to the identified organics taken as a whole. The calculation is performed using the carbon mass. So the full assumption for toxicity is that the unidentified chemicals' toxicity is adequately estimated by the identified organics' potency multiplied by the carbon mass of the unidentified portion. The calculation is given as (U.S. EPA, 1994, page 5):

$$Q_{i,adj} = Q_i \; x \; C_{TOC} \; / \quad C_i \qquad (6\text{-}2)$$

where

$Q_{i,adj}$ = adjusted emission rate of chemical i

Q_i = emission rate of chemical i

C_i = stack concentration of chemical i (carbon basis)

C_{TOC} = stack concentration of total organic carbon

The risk assessment is then based on the adjusted stack emission rates for each of the identified organic compounds. No adjustment is made for the metals emissions.

6.2.4.2. Method 2: Assume All Unidentified Organics Are Carcinogens — The main assumption is that all the unidentified organic chemicals are carcinogens with a combined potency similar to the average of the identified carcinogens on EPA's PIC list. This option was specifically developed because of the assumption that voluntarily identified compounds (i.e., those not on the PIC list) would most likely be noncarcinogens or low potency carcinogens. The calculation is given as (U.S. EPA, 1994, page 6):

$$Qcp_{i,adj} = Qcp_i x(C_{TOC} - \Sigma Cn_j - \Sigma Ccn_k) / \Sigma Ccp_i \qquad (6\text{-}3)$$

where

$Qcp_{i,adj}$ = adjusted emission rate of PIC list carcinogenic chemical i

Qcp_i = emission rate of PIC list carcinogenic chemical i

Ccp_i = stack concentration of PIC list carcinogenic chemical i (carbon basis)

Cn_j = stack concentration of noncarcinogenic chemical j (carbon basis)

Ccn_k = stack concentration of non-PIC list carcinogenic chemical k (carbon basis)

C_{TOC} = stack concentration of total organic carbon

The risk assessment is then based on the adjusted stack emission rates for each of the identified organic carcinogens on the PIC list and the measured (unadjusted) emissions for the organic carcinogens not on the PIC list, along with the organic noncarcinogens.

Other estimation procedures are possible for mixtures of other types of chemicals. Whenever toxicity or exposure of unidentified chemicals in the mixture is estimated from information on the identified component chemicals, the supporting evidence for the estimation must be clearly stated. The assessment should not presume that the similarity arguments given above for organics would always lead to an overestimate of the mixture risk. Unidentified chemicals, even degradation products, can be more toxic than the parent component chemicals. One option in the risk characterization is to present two risk estimates: one with only the identified chemicals, and one with both the identified and estimated chemicals.

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APPENDIX A

PHARMACOKINETICS

PHARMACOKINETIC/PHARMACODYNAMIC MODELING

The last two decades have seen great strides in our ability to assess the health risks of chemicals present in our air, water and food. Our ever-growing scientific databases are increasing our understanding of the dose-response toxicity of individual chemicals and are permitting better predictions of health effects. However, we are now reaching the point at which we can, and must, increase the complexity of our calculations and incorporate chemical-chemical interactions into our risk assessment analyses.

Although single-compound exposures are possible, in most instances, contaminant chemicals are present in our environment as mixtures. Some of these mixtures are relatively well defined, such as coke oven emissions and diesel exhaust. Other mixtures, such as those released from old disposal sites, are highly variable, complex, and largely undefined. As there is a considerable body of literature indicating that chemical-chemical interactions occur, factors that influence the toxicity of the chemicals in mixtures must be better understood if they are to be effectively incorporated into our health risk assessments (U.S. EPA, 1986).

In theory, there are many ways in which one chemical could alter the toxicity of another. Two chemicals could directly interact to form a new compound, or there may be changes in the intestinal absorption of the chemicals. Absorption could be altered through competition for membrane-binding sites or by the induction of a transport process. Plasma transport, tissue accumulation, and elimination processes could also be altered through competition or interference mechanisms, e.g. binding to metallothionein. Cellular metabolism and intracellular effects may be

modified either directly through competition for receptor- or enzyme-binding sites or indirectly by the induction or depression of metabolizing enzymes and/or other detoxification mechanisms, such as cellular glutathione levels.

Assessment of the health impacts of single chemicals or chemical mixtures present in our environment is an important problem. Although we have made progress in recent years by establishing "safe" concentrations and exposure conditions for many individual chemicals, related information for the same chemicals in mixtures is, largely, unavailable. Our challenge is to accurately evaluate the risk posed by exposure to multiple chemicals as compared to exposures to individual chemicals. This will occur only with a solid understanding of the mechanisms of toxicity of chemical agents and the factors that control their absorption, metabolism, distribution, and elimination.

Chemical interactions can be divided into two major categories: i.e., those resulting from toxicokinetic and those resulting from toxicodynamic mechanisms. Toxicokinetic mechanisms of interaction involve alterations in metabolism or disposition of a toxic chemical. These interactions can be mediated by the induction or inhibition of enzymes involved in xenobiotic activation and detoxification. Toxicodynamic mechanisms include interactions which do not directly affect the metabolism or disposition of a xenobiotic, but affect a tissue's response or susceptibility to toxic injury. Mechanisms of toxicodynamic interactions include, among others, depletion or induction of protective factors, alterations in tissue repair, changes in hemodynamics, immunomodulation. Sauer and Sipes (1995) have reported toxicodynamic action between all-*trans*-retinol and other chemicals which involves the alteration of chemical-induced tissue injury by the modulation of inflammatory cell activity.

Retinol pretreatment in this study provided protection against pulmonary toxicity induced by 2-nitronaphthalene and paraquat by suppressing the inflammatory response. The investigators looked at effects on liver for the combination of retinol and 2-nitronaphthalene. With this target organ, they observed a potentiation of toxicity, rather than protection as seen in the lung. A subsequent experiment indicated that retinol-induced activation of Kupffer cell function was a major contributing factor in the lung. The selective destruction of Kupffer cells by gadolinium pretreatment protected rats against the potentiation induced by retinol. From these studies, it is clear that it can be difficult to predict interactions from one organ to another, let alone from species to species. Likewise, results described indicate that *in vitro* studies alone would have been of limited use in describing the range of effects observed in the intact animal with these combinations.

Glutathione (GSH) plays a critical role in detoxifying many chemicals and its depletion within cells has long been known to increase the risk of chemical toxicity. Jones et al. (1995) have provided information on factors that regulate GSH status in humans, including gender, age, race and dietary habits that could affect the risk of exposure. GSH levels in human plasma are highly variable and potentially be a marker of susceptibility. Because of GSH's central role in detoxifying many chemicals, therapeutic manipulation of GSH levels may afford extra protection that could reduce the risks of exposure to complex mixtures.

The utility of physiologically-based pharmacokinetic (PB-PK) modeling in predicting the consequences of exposure to multiple solvents has been demonstrated by Krishnan and Pelekis (1995). The authors used PB-PK models and existing datasets to predict the effect of multiple solvent exposure on carboxyhemoglobin formation from dichloromethane. The interaction involved the hepatic metabolism of the various solvents by one isozyme of cytochrome P450

(CYP2E1) and the effect of one metabolite, CO, on hemoglobin. Their predictions highlighted the need to understand the disposition of chemicals and mechanisms of toxicity in order to effectively use PB-PK in risk assessment.

This modeling exercise suggested that, with competitive metabolic inhibition mechanism, the threshold for the appearance of binary chemical interactions will follow a downward trend with increasing number of substrates or structurally-similar substances in a mixture. The use of this kind of mechanistic model, along with data from descriptive chemical interaction studies, could form the very basis of mechanistic risk assessment methods for complex chemical mixtures.

Several studies on toxic interactions have been published to date; the quantitative aspect of toxicokinetic/toxicodynamic mechanism of interactions, however, has only been elucidated for a few chemical pairs (Krishnan and Brodeur, 1991). One approach to the problem in assessing risk in the context of a complex mixture would be to develop biologically-based dosimetry and toxicity models, such that multiple interactions can be simultaneously distinguished and systematically analyzed at any level of complexity. Physiologically based-pharmacokinetic and pharmacodynamic modeling (PB-PK/PD) may therefore be considered as a viable approach. Tardiff et al. (1997) developed a PB-PK model for a ternary mixture of alkyl benzenes in rats and humans. Model simulations and experimental data obtained in humans indicated that exposure to atmospheric concentrations of the alkyl benzenes that remained within the permissible concentrations (TLVs) for a mixture would not result in biologically significant modifications of their pharmacokinetics. This study demonstrated the utility of PB-PK models in the prediction of the kinetics of components of chemical mixtures, by accounting for mechanisms of binary chemical interactions.

The linkage of two of the most challenging areas in toxicology today, (1) PB-PK/PD and statistical/mathematical modeling and (2) experimental toxicology of chemical mixtures, will have immense potential in application to risk assessment for chemical mixtures. Figure A-1 represents the possible application of combined PB-PK/PD modeling to chemical mixtures and the development of innovative risk assessment methodologies for chemical mixtures. El-Masri et al. (1996) attempted to couple PB-PK/PD and other experimental toxicology with isobolographic analysis and/or response surface methodology for the modeling and analysis of toxicologic interactions. With the aid of such techniques as Monte Carlo simulation, one may then estimate tissue dosimetry at the pharmacokinetic and pharmacodynamic levels. Using these tissue values as benchmark doses, human risk assessment of chemical mixtures may possibly be carried out with quantification of the uncertainty.

PHARMACOKINETIC PRINCIPLES: CHEMICAL MIXTURES

Environmental exposures to naturally-occurring and artificially-produced substances indicate that most exposures are to mixtures of chemicals. Exposure to single chemicals occurs in the context of simultaneous exposure. When therapeutic agents are taken with the intent to produce a certain pharmacological effect, other chemicals present at the time of their disposition may modulate processes of absorption, tissue distribution, metabolism, or excretion so as to alter the shape of the dose-effect relationship. Toxicokinetic interactions may influence the relationship between administered dose and the dose delivered to the target site(s). This forces the distinction between toxicokinetic interactions and toxicodynamic interactions. Toxicologic agents, or pharmacologic agents administered at doses at which they exert other than their intended effects, more than likely will interact with a variety of receptor sites, reversibly or irreversibly. Metabolites, in particular, although they may be formed in very small amounts,

***A Priori* PB-PK/PD Modeling**

Model Directed Focused Experiments/
Efficient Experimental Designs

PB-PK/PD
Integrated +
Toxicity
Model

Isobolographic Analysis
Response Surface Methodology
Monte Carlo Simulation

Predictive and Alternative Toxicology/
Target Tissue Dosimetry

Innovative Risk Assessment
Methodologies

FIGURE A-1

The Possible Application of Combined PB-PK/PD Modeling to Chemical Mixtures

may not move from the tissue or even the intracellular site where they were produced. Given this broad spectrum of mechanisms of action, it is not surprising that toxicodynamic models of action and interaction are less fully developed than toxicokinetic models. The interactions among chemicals may occur at any point during absorption or disposition of the chemical components of the mixture. O'Flaherty (1989) reviewed these mechanisms of kinetic interaction during absorption and elimination; the following discussions summarize this review and include other pertinent information available in the current literature.

Absorption

Gastrointestinal

Gastrointestinal transit time may be affected by the constituents of a mixture. For example, absorption may be higher or lower depending on transit time. Although some lipophilic substances, such as paraffin oil and triglycerides, do not affect uptake, others, such as lipophilic substances possessing hydrophilic groups such as oleic acid and oleyl alcohol, alter absorption into the outermost layer of the glandular mucosa. When both hydrophilic and lipophilic groups are present in the solvent with dominant hydrophilic characteristic, an administered compound readily penetrates into the stomach wall (Ekwall et al., 1951). Many other factors, e.g. acid-base balance in the gastrointestinal lumen, gut mobility and blood flow, also affect the absorption of many xenobiotics. From a practical point of view, it is important to differentiate between interactions that alter the rate of absorption from those that affect the amount of xenobiotic absorbed. Kristensen (1976) has reported that rate of absorption contributing to a longer plasma half-life may be needed to maintain a steady-state concentration of certain drugs, e.g., antihypertensive drugs, whereas a shorter plasma half-life, or attainment of higher unbound

plasma levels of an active drug (e.g., digitoxin, oubain) because of rapid passage across gut, may be important when a quick onset of drug effect is desired.

The competitive binding of metals to macromolecules can influence their intestinal absorption, plasma transfer, tissue uptake, intracellular binding, and site-specific toxic effects. The following discussion cites examples of such interactions. Although many have not been studied in detail, it is possible that we have a lot to discover in this area.

The intestinal absorption and tissue accumulation of most toxic metals are influenced, to a large extent, by the concentration of essential trace metals present in one's diet (Eisenhans et al., 1991). The intestinal uptake of cadmium (Cd), for example, is significantly increased under conditions of iron (Fe), zinc (Zn) and calcium (Ca) deficiency (Hoadley and Johnson, 1987). Dietary Zn alters lead (Pb) toxicity, as evidenced by decreased Pb absorption, lower blood and tissue Pb levels, and decreased inhibition of the Pb-sensitive enzyme aminolevulenic acid dehydrase (ALAD) (Cerklewski and Forbes, 1976) under conditions of elevated dietary Zn exposure.

The mechanisms underlying these effects undoubtedly involve multiple mechanisms. Some of these interactions occur through competition of the metal ions for membrane transport systems, in a manner similar to that described by Blazka and Shaikh (1992) for Cd. These investigators have found that Cd uptake by rat hepatocytes occurs through a sulfhydryl (SH)-containing transport process that is inhibited by concomitant exposure to copper (Cu), iron and zinc. Thus, the relative extracellular concentrations of these ions will be an important determinant of Cd uptake and accumulation. *In vivo* studies of hepatic Cd, Cu and Zn uptake and accumulation suggest that influx and efflux of metal ions are both important determinants of final tissue metal concentrations (Suzuki et al., 1991).

In addition to mediating cellular toxicity in target organs, metallothionein (MT) in intestinal cells alters the absorption of metals from dietary sources. Richards and Cousins (1975) have proposed that MT regulates Zn absorption by chelating Zn ions in intestinal cells, preventing their transfer across the basal membrane into the circulatory system. This proposed function of MT is supported by the observation that intestinal MT concentrations are inversely proportional to Zn absorption (Bremner, 1993). The binding of Cd ions to MT in the intestine similarly decreases Cd absorption. Foulkes (1991) has demonstrated that pretreatment of animals with Zn at levels that increase mucosal MT content causes a decrease in Cd transport across the intestinal lumen.

Adsorption can reduce bioavailability from the gastrointestinal tract. Prescott (1969) demonstrated that the salts of Ca, Fe or magnesium (Mg) may interact with drugs in the intestine to produce insoluble and nonabsorbable complexes. For example, calcium phosphate filler markedly reduces the absorption of tetracycline. In addition to calcium salts, Fe and aluminum (Al) ions also form insoluble chelate complexes with tetracycline. These interactions, of potential clinical significance, are avoidable if the drugs are given in properly spaced time intervals (Neuvonen, 1976).

Pulmonary

Gaseous and particulate phases of an inhaled chemical mixture may play different functional role inducing or reducing pulmonary/systemic toxicity. For example, formaldehyde can stimulate mucocilliary function at low concentrations, while it inhibits mucocilliary function after prolonged exposure at high concentrations (Morgan et al., 1984). Gaseous and particulate phases of cigarette smoke are cilia toxic and at sustained high levels can cause impairment of tracheobronchial clearance. Low, brief exposures, however, actually appear to speed up lower

bronchial transport. In occupational settings, however, chronic lower exposures than those associated with ambient air may significantly interfere with pulmonary clearance and may produce a variety of toxicological events uncommon to the individual constituents of the mixture (Albert et al., 1975; Ferin and Leach, 1973).

Airborne particulates, when adsorbed to chemical constituents of gases/vapors, may influence the degree of absorption from the lung. Other factors, particle size, length and binding affinity, can also play a significant role in pulmonary absorption/retention. Henry and Kaufman (1973) suggested that the ability of benzo[a]pyrene (B[a]P) to be eluted for its particulate adsorption sites might be an important determinant of its biological activity. Creasia et al. (1976) reported that B[a]P adsorbed to the larger carbon particles was cleared with the particles themselves. Since the half-times of the large and small particles were similar, B[a]P adsorbed to the smaller carbon particles was cleared about four times as fast as the particles from the mouse lung.

Dermal

Despite lack of sufficient quantitative information, solvent effects on qualitative absorption for dermal route are well characterized. Within a limited range at least, partition coefficients calculated for solubilities in skin and in various solvents appear to correlate with permeability coefficients for penetration into the skin for those solvents (Sloan et al., 1986).

Although an adequate amount of information is known about the uptake of several classes of neat chemicals (as liquids) through human skin, more needs to be known about the effects of media on dermal uptake. In the workplace, employees are frequently exposed to liquid chemicals but environmental exposure almost never involves exposure to neat substances. For example, residents may be exposed to contaminated dust which has been transported through open

windows. Children are exposed to soils that have contaminants from particulate emissions from cars, smelters, foundries, incinerators, or other processes that have been deposited on yards and playgrounds. Adults and children can also be exposed to several organic contaminants in water during showering or swimming.

Information on the neat chemical is helpful in understanding the dermal uptake of chemicals bound to soil, dust, sludge, sediment, paint, etc., but there are other factors that should also be considered. The best approach for mixtures assessments is to conduct specific tests with thecontaminated chemical on laboratory animals or using *in vitro* technologies. Since relatively low concentrations of the chemical are typical in the environment and high concentrations are used in laboratory studies, an extrapolation to environmental levels is often necessary. Other factors such as the duration of contact, integrity of the skin, and the chemical properties of the agent must ultimately be considered in the risk assessment.

Progress continues to be made to allow risk assessors to make fairly reasonable estimates of the uptake of chemicals in soil. The development of models which can predict dermal bioavailability and account for media effects would represent a signficant step forward. The role of concentration on the rate of dermal uptake is an area that serves further study. Work conducted thus far suggests that the uptake will depend on the characteristics of the media (% organics, particle size in soil, etc.) and the properties of the contaminant (lipophilicity, temperature). These parameters need to be quantified and a general model developed. The work of McKone (1990) represents an important step in this direction.

Elimination

Metabolism of one chemical may deplete reserves of a cofactor required for metabolism of another chemical, reducing exposure to metabolites of the second chemical or shifting the relative

magnitudes of exposure to products of competing metabolic pathways. Induction of metabolizing enzymes, often those of cytochrome P-450-dependent mixed-function oxidase (MFO) system, can alter the relative magnitudes of parallel pathways of metabolism as well as increase the rate of magnitude of total metabolic production (O'Flaherty, 1989).

Anderson et al. (1987), while developing a PB-PK model, considered the interaction between 1,1-dichloroethylene (1,1-DCE) and trichloroethylene (TCE) that are metabolized by the same enzyme system. In this study rats were exposed to these chemicals via inhalation. When the chemicals reached dynamic steady states among the tissues and between blood and alveolar air, the rate of loss of 1,1-DCE was found to be sharply reduced in the presence of TCE. Of the several modeled mechanisms of interaction, competitive interaction gave the most successful predictions. This lead to the development of a co-exposure model with competitive interaction to predict the kinetic behavior of either compound in the presence of the other. The success with which this was done was illustrated by a good concordance between predicted and observed chamber concentrations of 1,1-DCE without and with coexposure to TCE.

Induction of metabolizing enzyme may produce different effects on metabolic rates which could reduce integrated exposure to the parent chemical by increasing its rate of metabolism. For instance, caffeine metabolism has been modeled as a capacity-limited process giving rise to the three monitored metabolites (York et al., 1987). Elimination of the metabolites was assumed to be first-order, an assumption justified by the observations that at no time did the concentration of any metabolite exceed 1/10 of the maximum caffeine concentration and the caffeine itself; indicating moderated capacity-limited behavior. Integrated exposure to caffeine, as expected, decreased as a consequence of induction of caffeine metabolism; however, integrated exposure to

individual monodemethylated metabolites was also decreased by induction of caffeine metabolism. This could probably be explained by consideration of a process of caffeine elimination.

The toxicity of many organic chemicals is influenced by the action of mixed-function oxidases (MFOs) and phase II biotransformation enzymes that catalyze their metabolism to more hydrophilic forms in preparation for excretion. Because the synthesis of many of these enzymes is affected by the chemicals they metabolize, multiple mechanisms may be involved in the chemical interactions involving these enzyme systems (Kedderis, 1990). For example, an inhibition of toxicity can occur when the metabolism of one chemical to its more toxic form is prevented by the preferential metabolism of another compound, or when one chemical induces an MFO enzyme system that can catalyze the transformation of a second chemical to a less toxic form. On the other hand, enhancement of toxicity can occur when the enzyme that bioactivates a chemical has been previously induced in a cell by exposure to a second compound. Thus, the toxicity of each individual chemical, in each situation, will depend on which biotransformation enzymes have been induced, the relative affinity of each chemical for the available enzymes, and the relative toxicity of the metabolized forms of the chemicals compared with the parent compounds.

There are numerous examples of chemical interactions in experimental animals that have their genesis in biotransformation. Chemicals such as piperonal butoxide and proadifen (SK&F 525A), which inhibit MFO enzymes, decrease the hepatic toxicity of such compounds as acetaminophen, bromobenzene, and cocaine, which require activation for toxicity (Thompson et al., 1979). Increased toxicity can also occur when MFO enzymes are inhibited if a compound is normally converted by these enzymes to a less toxic form. This appears to be the basis for the

increased nephrotoxicity of cyclosporine that occurs following cotreatment with compounds such as ketoconazole, methyltestosterone, and erythromycin (Moller and Ekelund, 1985).

In addition, the timing of the multiple-chemical exposures and the doses used can affect the outcome of an interaction study (Plaa and Vezina, 1990). Plaa and Hewitt (1982), for example, demonstrated that the magnitude of hepatotoxicity caused by chloroform varied over 100-fold when a second chemical, 2,5-hexanedione, was administered 10 versus 50 hours before the chloroform. Also, MacDonald et al. (1982) have shown that, whereas low doses of acetone enhanced the toxicity of haloethanes such as trichloroethane, high doses reduced toxicity. Thus, nonlinear or biphasic response curves for individual chemicals will lead to nonlinear and biphasic interactive effects that must be considered in predictive studies.

TABLE 2-4

Methods for Whole Mixture Data

Type of Available Data	Type of Assessment	Procedure (Sections)	Number of Components	Applicability; Ease of Use	Assumptions and Features of Method	Strategy of Method
Data on mixture of concern	Dose-response assessment for any toxic endpoint; examples on IRIS (U.S. EPA, 1999)	Mixture of concern (2.3., 3.1.)	Any	Data rarely available; simple to calculate; uncertainties must be explained	Composition of test mixture functionally the same as field mixture; test data cover all sensitive endpoints	Estimate dose-response value directly from data on mixture of concern
Data on similar mixtures; no data on mixture of concern	Dose-response assessment for any toxic endpoint; same procedure as above	Similar mixture (2.4., 3.1)	Any	Data limited; simple to calculate; uncertainties must be explained	Similarity judgment across mixtures; plus above	Estimate dose-response value using similar mixture data as a surrogate for mixture of concern
Data on similar mixtures; limited data on mixture of concern	Dose-response assessment for cancer, genetic toxicity; used for combustion mixtures (Lewtas, 1985, 1988; Nesnow, 1990)	Comparative potency (2.4, 3.2.)	Any	Data limited; short-term assay data required; data intensive; requires statistical modeling	Requires short-term data and at least one data point from a chronic *in vivo* study; assumes the potency change across assays is the same for all similar mixtures; plus above	Estimate dose-response value using relationships across similar mixtures and similar assays to extrapolate value for mixture of concern

TABLE 2-5

Methods for Component Data

Type of Available Data	Type of Assessment	Procedure (Sections)	Number of Components	Applicability; Ease of Use	Assumptions and Features of Method	Strategy of Method
Toxicity and exposure data	Risk characterization for any toxic endpoint; used in Superfund site assessments (U.S. EPA, 1989a)	Hazard Index (HI) (2.5., 4.2.) Reference value (RfD, RfC) (4.5)	Limited by data quality, similarity of mode of action, accuracy of exposure data	Good dose-response data estimates, e.g., IRIS data; exposure data at relatively low levels; easy to calculate	Dose addition (toxicologic similarity) at exposure concentrations near NOAELs; can be improved by replacing RfD in HI by TTD or human BMD	Scale exposure concentrations by a measure of relative potency across chemicals with similar mode of action; add scaled doses; provides an indicator of mixture risk
Toxicity and exposure data; one well studied chemical; common mode of action established	Dose-response assessment for any toxic endpoint; used for dioxins and furans (U.S. EPA, 1989b)	Toxicity Equivalence Factor (2.5., 6.1.)	Limited by data quality, similarity	Rare data; restricted by strong similarity, so few chemical classes will qualify; applied to all endpoints and all routes; complicated to use; data intensive	Requires strong degree of toxicologic similarity based on dose addition; judgment of relative potency factor	Scale exposure concentrations relative to potency of an index chemical; add scaled doses; use dose-response curve of index chemical to estimate dose-response value for the total mixture dose

TABLE 2-5 cont.

Type of Available Data	Type of Assessment	Procedure (Sections)	Number of Components	Applicability; Ease of Use	Assumptions and Features of Method	Strategy of Method
Toxicity and exposure data; missing toxicity data on some components; mode of action not proven	Dose-response assessment for any toxic endpoint; new procedure	Relative Potency Factor (2.5., 6.1.)	Limited by data quality, similarity; may not have data from all routes of exposure of interest	Some data, restricted by similarity; restricted to specific conditions; complicated to use	Requires toxicologic similarity, but for specific conditions (endpoint, route, duration); based on dose addition; judgment of relative potency factor	Scale exposure concentrations relative to potency of an index chemical; add scaled doses; use dose-response curve of index chemical to estimate dose-response value for the total mixture dose
Toxicity and exposure data; interactions data on at least one pair of components	Risk characterization for any noncancer endpoint; new procedure	WOE Toxicologic Similarity (2.5., 4.4.)	Limited by data quality	Limited interactions data; complicated to use	Assumes binary interactions are most important; model with relative proportions untested; assumes interaction magnitude not dose-dependent	Scale exposure concentrations by a measure of relative potency across chemicals with similar toxicity; modify this term with data on binary interactions; add scaled/modified doses; provides an indicator of mixture risk
Toxicity and exposure data; interactions data on at least one pair of components	Qualitative risk characterization of cancer interactions; new procedure	WOE Cancer (2.5., 5.2.)	Limited by data quality, availability	Limited interactions data; complicated to use	Assumes binary interactions are most important	Uses data base information to provide a qualitative modification of risk estimates made using response addition

TABLE 2-5 cont.

Type of Available Data	Type of Assessment	Procedure (Sections)	Number of Components	Applicability; Ease of Use	Assumptions and Features of Method	Strategy of Method
Toxicity and exposure data	Risk characterization for any toxic endpoint; used extensively for cancer; used for Superfund site assessments (U.S. EPA, 1989a)	Response Addition (2.5., 5.1.)	Restricted to independence of action; slight overestimate of mixtures risk when adding upper bounds	Used at low levels of chemicals; good data, e.g., IRIS; easy to use	Assumes functional independence of action; assumes interactions not significant at low doses; potency estimates vary in quality	Dose-response curves are used to estimate component risks for a given exposure; risks are added to yield a risk estimate for the total mixture for the specific exposure
Toxicity and exposure data	Risk characterization for any endpoint; used for cancer assessment of PCBs (U.S. EPA, 1996a)	Geographic Site-Specific Assessments (2.5., 6.2.)	Limited by data quality, similarity	Some data restricted by similarity; restricted to specific conditions; complicated to use; data intensive	Requires assumptions about fate and transport for groups of chemicals	Toxicity data on the commercial mixture are used to estimate a range of toxicity values that are then adjusted for alterations in mixture composition due to environmental factors to produce a risk estimate for the total mixture

TABLE 4-1

Example Application of the Target-organ Toxicity Dose

Chemical	Hepatic TTD	Renal TTD	Reproductive TTD	Oral Exposure (mg/kg per day)	RfD (mg/kg per day)	HQ	Critical Effect
Acetone	1.00E-01 RfD	1.00E-01 RfD	NA	4.E-02	1.E-01	0.40	Renal, Hepatic
Chloroform	1.E-02 RfD	1.E-01 TTD	NA	5.E-03	1.E-02	0.50	Hepatic
Dibutyl phthalate	NA	NA	2.E-01 TTD	8.E-02	1.E-01	0.80	Incr. mortality
Diethyl phthalate	NA	NA	5.E+00 TTD	1.E+00	8.E-01	1.25	Growth
Di(2-ethyl-hexyl) phthalate	2.E-02 RfD	2.E-02 RS	5.E-02 TTD	1.E-02	2.E-02	0.60	Hepatic
Phenol	NA	2.E+00 TTD	NA	3.E-01	6.E-01	0.50	Developmental
HI-RfD	1.5	2.0	2.7				
HI-TTD	1.5	1.2	0.8				

In the TTD columns, the source of the value is coded as:

TTD= new TTD developed for this effect.
RfD= this is the critical effect, so the TTD=RfD.
RS= insufficient data for a TTD, so RfD used as a surrogate.

TTDs and RfDs are from Mumtaz et al., 1997. Exposure levels (dose) are set for illustration only.

TABLE 4-3

Default Weighting Factors for the Modified Weight-of-Evidence

Category	Description	Direction	
		Greater than Additive	Less than Additive
I.	The interaction has been shown to be relevant to human health effects and the direction of the interaction is unequivocal.	1.0	-1.0
II.	The direction of the interaction has been demonstrated *in vivo* in an appropriate animal model and the relevance to potential human health effects is likely.	0.75	-0.5
III.	An interaction in a particular direction is plausible but the evidence supporting the interaction and its relevance to human health effects is weak.	0.50	0.0
IV.	The assumption of additivity has been demonstrated or must be accepted.	0.0	0.0