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Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures

RISK ASSESSMENT FORUM

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Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures

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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 Sciences
NOAEL	No-Observed-Adverse-Effect Level
NRC	National Research Council

LIST OF ABBREVIATIONS (continued)

OSHA	Occupational Safety and Health Administration
PAH	Polycyclic Aromatic Hydrocarbon
PBPK	Physiologically Based Pharmacokinetics
PBPK/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

PREFACE

The U.S. EPA's Risk Assessment Forum (Forum) is publishing the *Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures* as a supplement to the EPA's *Guidelines for the Health Risk Assessment of Chemical Mixtures (Guidelines)*(U.S. EPA, 1986) (Appendix A). 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 principles and concepts put forth in the Guidelines remain in effect. However, where the Guidelines describe broad principles and include few specific procedures, the present guidance is a supplement that is intended to provide more detail on these principles and procedures.

To address concerns over health risks from multichemical exposures, the U.S. Environmental Protection Agency published the *Guidelines for the Health Risk Assessment of Chemical Mixtures* in 1986 (U.S. EPA, 1986) (Appendix A). The Guidelines describe broad concepts related to mixture exposure and toxicity and include few specific procedures. In 1989 EPA published guidance for the Superfund program on hazardous waste that gave practical steps for conducting a mixtures risk assessment (U.S. EPA, 1989a). Also in 1989, 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 (U.S. EPA, 1989b). In 1990, 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. 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, 1990b).

This supplementary guidance document is a result of several influences. Because the science of environmental risk assessment has continued to evolve and EPA has learned from an array of experiences, the Agency charged the Risk Assessment Forum with developing guidance on challenging issues such as cumulative risk assessment. Part of the Forum's response to this charge was to establish a Technical Panel to ensure that the advances in the area of chemical mixtures health risk assessment are reflected in Agency-wide guidance materials. Through the evaluation of waste sites for mixtures risks it has become apparent that the exposure scenarios for these sites are extremely diverse. Moreover, the quality and quantity of pertinent information available for risk assessment has varied considerably for different mixtures. Other Agency and external initiatives have influenced the development of the chemical mixtures supplementary guidance:

- The National Academy of Sciences has issued a recommendation to move away from single-chemical assessments. (NRC, 1994)

- In 1997, EPA's Science Policy Council issued a policy statement on cumulative risk assessment. This policy addressed the first step in the overall assessment process (i.e., problem formulation) (U.S. EPA, 1997a).

- Siting activities have raised the issue of multiple chemical exposures. Parties are concerned not only about what risks are associated with releases from a particular facility, but also the potential combined effects of exposures from other sources in the area.

- EPA's research strategy for 2000 and beyond emphasizes research on chemical mixtures.

When the 1986 Guidelines were published, the Agency recognized that the Guidelines would need to be updated as the science of chemical mixture assessment evolved. Research efforts were undertaken immediately and by 1988 Agency offices were discussing revision topics. By 1989, under the auspices of the Risk Assessment Forum, efforts were underway to revise the Guidelines. Updates to the Guidelines were reviewed in a June 1997 *Internal Risk Assessment Forum Review Draft of the Guidance on Health Risk Assessment of Chemical Mixtures*. The Technical Panel revised the document in accordance with comments received during the July 1997 review. In June 1998 the Forum sponsored an Agency review and colloquium. Over the next months the Technical Panel worked with commenters to address issues raised during the 1998 colloquium to prepare the document for external peer review. It was determined at this time that the broad principles and concepts put forth in the 1986 Guidelines remained applicable, but needed more detail. As a result it was determined that the document would supplement, and not replace the 1986 Guidelines. An external peer review was convened in May 1999. Twelve independent experts representing consulting, academia, industry, the U.S. Department of Health Agency for Toxic Substances and Disease Registry, and the TNO Nutritional and Food Research Institute of the Netherlands, reviewed the revised supplementary document dated April 1999. The experts provide comments that reflected their experience and expertise in toxicology, mechanistic and pharmacokinetic modeling, statistics, and risk assessment (risk assessment of chemical classes, of complex and unidentifiable mixtures, and of multi-chemical exposures at Superfund sites). Their comments are documented in the report entitled, *Report of the Peer Review Workshop on the Guidance for Conducting Health Risk*

Assessments of Chemical Mixtures (Eastern Research Group Inc., 1999). During the summer of 1999 the Technical Panel considered comments from the external experts and from the Forum in revising and reorganizing the supplementary document. This series of internal and external reviews has ensured that the supplementary guidance is consistent with related science and Agency guidance developments.

After an abbreviated overview of the background and scope, the *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* document puts forth the risk assessment paradigm for mixtures. This paradigm begins with problem formulation, then briefly discusses hazard identification, dose-response assessment, exposure, and risk characterization. The document is organized according to the type of data available to the risk assessor, ranging from data-rich to data-poor situations. (See Figure 2-1). Procedures are described for assessment using data on the mixture of concern, data on a toxicologically similar mixture, and data on the mixture component chemicals. The state of the 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. 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. The appendices contain definitions, a discussion on toxicologic interactions and pharmacokinetic models, and a reprint of the 1986 Guidelines.

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EXECUTIVE SUMMARY

This supplementary guidance document is organized according to the type of data available to the risk assessor, ranging from data rich to data poor situations. This organization reflects the approaches to chemical mixture risk assessment recommended in the 1986 *Guidelines for the Health Risk Assessment of Chemical Mixtures* (Appendix A). This document describes more detailed procedures for chemical mixture assessment using data on the mixture of concern, data on a toxicologically similar mixture, and data on the mixture component chemicals. The state-of-the-science varies dramatically for these three approaches. It is recommended that the risk assessor implement several of the approaches that are practical to apply and evaluate the range of health risk estimates that are produced.

This document suggests that the selection of a chemical mixture risk assessment method follows the outline in the flow chart shown in Figure 2-1, which begins with an assessment of data quality and then leads the risk assessor to selection of a method through evaluation of the available data. The major concerns for the user are whether the available data are on components or whole mixtures, whether the data are composed of either similar components or similar mixtures that can be thought of as acting by similar toxicologic processes, and whether the data may be grouped by emissions source, chemical structure, or biologic activity. Method-specific user fact sheets for quantitative risk assessment can be found in Sections 2.5 and 2.6 and are intended to provide a concise overview of each currently available method. These fact sheets provide the following information relative to the risk assessment approach:

- Type of Assessment
- Data Requirements
- Section(s)
- References
- Strategy of Method
- Ease of Use
- Assumptions
- Limitations
- Uncertainties

In Figure 2-1, an evaluation of the data may lead the user to decide that only a qualitative analysis should be performed. This generally occurs in cases where data quality is poor, inadequate quantitative data are available, data on a similar mixture cannot be classified as

"sufficiently similar" to the mixture of concern, exposures cannot be characterized with confidence, or method-specific assumptions about the toxicologic action of the mixture or of its components cannot be met. When this occurs, the risk assessor can still perform a qualitative assessment that characterizes the potential human health impacts from exposure to that mixture. Such a risk characterization should discuss each element of the risk assessment paradigm, including available information on the mixture itself, on its components, and on potential interactions among the components. Any information on fate and transport of the mixture that would affect its final composition at the time of exposure should be noted.

The assessment of chemical mixtures is an area of active scientific investigation. As new information relevant to health risk from exposure to chemical mixtures becomes available, additional guidance documents will be published.

1. INTRODUCTION

1.1. BACKGROUND

Although 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, a mixture 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 into 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. Environmental Protection Agency, hereafter referred to as EPA, issued *Guidelines for the Health Risk Assessment of Chemical Mixtures* in 1986 (U.S. EPA, 1986) (Appendix A). Those Guidelines described broad concepts related to mixture exposure and toxicity and included few specific procedures. In 1989, EPA published guidance for the Superfund program on hazardous waste that gave practical steps for conducting a mixtures risk assessment (U.S. EPA, 1989a). Also in 1989, 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 (U.S. EPA, 1989b). In 1990, 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 guidance documents 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 similar mixture, and 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.

Mixture 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 should 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 addresses only 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. 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 to modify a Hazard Index (HI), 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. Some 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 EPA generally follow the paradigm established by the National Academy of Sciences (NRC, 1983). This paradigm describes a group of interconnected processes for performing a risk assessment that include hazard identification, dose-response assessment, exposure assessment, and risk characterization. These four parts of the paradigm are used as the foundation for the procedures presented in this guidance. Preamble to all is problem formulation, which is defined in EPA's (1998a) Ecological Risk Assessment Guidelines as "a process for generating and evaluating preliminary hypotheses about why...effects have occurred or may occur." This EPA guidance for assessing risks from exposures to chemical mixtures begins with problem formulation as the initial step; much of the information about this key step has been adapted from the Ecological Risk Assessment Guidelines, and the reader is referred to Chapter 3 of that document for a more comprehensive discussion (U.S. EPA, 1998a).

2.1.1. Problem Formulation

Problem formulation, which provides the foundation for the entire risk assessment, consists of three initial steps: (1) evaluate the nature of the problem, (2) define the objectives of the risk assessment, and (3) develop a data analysis and risk characterization plan. The quality, quantity, and pertinence of information will determine the course of problem formulation. It concludes with three products: (1) selection of assessment endpoints, (2) review of the conceptual models that describe the relationship between exposure to a mixture of chemicals and risk, and (3) adjusting the analytic plan. (The pertinence of the information that is available at the outset of the assessment, in combination with the assessment objectives, will identify the types of information that should be collected through the analytic plan.) Ideally, the problem is formulated jointly by risk analysts and risk managers. While the steps and outcomes associated with problem formulation are presented separately, experiences from ecological applications and Superfund site assessments show the process to be frequently interactive and iterative rather than linear.

2.1.2. Hazard Identification and Dose-Response Assessment

In *hazard identification*, available data on biological endpoints are used 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 from animal studies and occasionally from

human studies) are used to estimate the amount of material that may produce a given effect in humans. The risk assessor may calculate a quantitative dose-response relationship usable for low-dose exposure, often by applying mathematical models to the data.

2.1.3. Exposure

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, as well as media, pathways, and routes of exposure, 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.

2.1.4. Risk Characterization and Uncertainty

Risk characterization is the integrating step in the risk assessment process that summarizes assessments of effects on human health and ecosystems and assessments of exposure from multiple environmental media, identifies human subpopulations or ecological species at elevated risk, combines these assessments into characterizations of human and ecological risk, and describes the uncertainty and variability in these characterizations. In March 1995, the Administrator of EPA issued the *Policy for Risk Characterization at the U.S. Environmental Protection Agency* (U.S. EPA, 1995). 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* (NRC, 1994), produced primarily for implementation of the 1990 Amendment to the Clean Air Act but applicable more generally, 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.

2.1.5. Incorporating the Paradigm Into Mixtures Guidance

EPA regularly publishes guidelines to provide for consistency of application and communication of risk assessment. Guidelines were published in 1986 on assessment of the

following areas: exposure, developmental effects, germ cell mutagenicity, carcinogenic effects, and chemical mixtures (U.S. EPA, 1986, 1987). Since that time, the Agency has revised some of these Guidelines and also published new Guidelines. These include Guidelines on developmental toxicity (U.S. EPA, 1991a), exposure assessment (U.S. EPA, 1992), cancer (proposed revisions) (U.S. EPA, 1996a), reproductive toxicity (U.S. EPA, 1996c), and neurotoxicity (U.S. EPA, 1998b). All of the EPA guidelines for human health risk assessment incorporate the four parts of the NAS paradigm.

For this supplemental guidance on the risk assessment of chemical mixtures, the four parts of the paradigm are 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. For mixtures, 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 part of the paradigm. The methods are organized instead according to the type of available data. In general, risk characterization takes into account both human health and ecological effects, and also assesses multiroute exposures from multiple environmental media. This guidance focuses only on the human health risk assessment for chemical mixtures and only discusses multiroute exposures in terms of conversions from dermal to oral.

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) (Appendix A) recommend three approaches to quantitative health risk assessment of 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 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 collection, they provide the risk assessor with a number of reasonable options for evaluating risk for chemical mixtures.

2.2.2. Steps for Selection

This guidance suggests that the selection of a chemical mixture risk assessment method follow the outline in the flow chart shown in Figure 2-1, which begins with an assessment of data quality and then leads the risk assessor to selection of a method through evaluation of the available data. The major concerns for the user are whether the available data are on components or whole mixtures, whether the data are composed of either similar components or similar mixtures that can be thought of as acting by similar toxicologic processes, whether the mixture components act by the same mode of action or are functionally independent, or whether the data may be grouped by emissions source, chemical structure, or biologic activity.

This document is organized around the decision points in Figure 2-1, so that the user can refer to specific sections and find guidance on the issues to consider when working through the flow chart. Appendix B also offers the user a number of definitions to help clarify the terminology that is unique to chemical mixtures risk assessment. Table B-1 presents chemical mixture 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. Table B-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.

Method-specific user fact-sheets in Sections 2.5 and 2.6 are intended to provide a concise overview of each currently available method. These fact-sheets provide the following information relative to the risk assessment approach:

- Type of Assessment: distinguishes whether the approach is a dose-response assessment or whether it combines dose response and exposure information to perform a risk characterization.

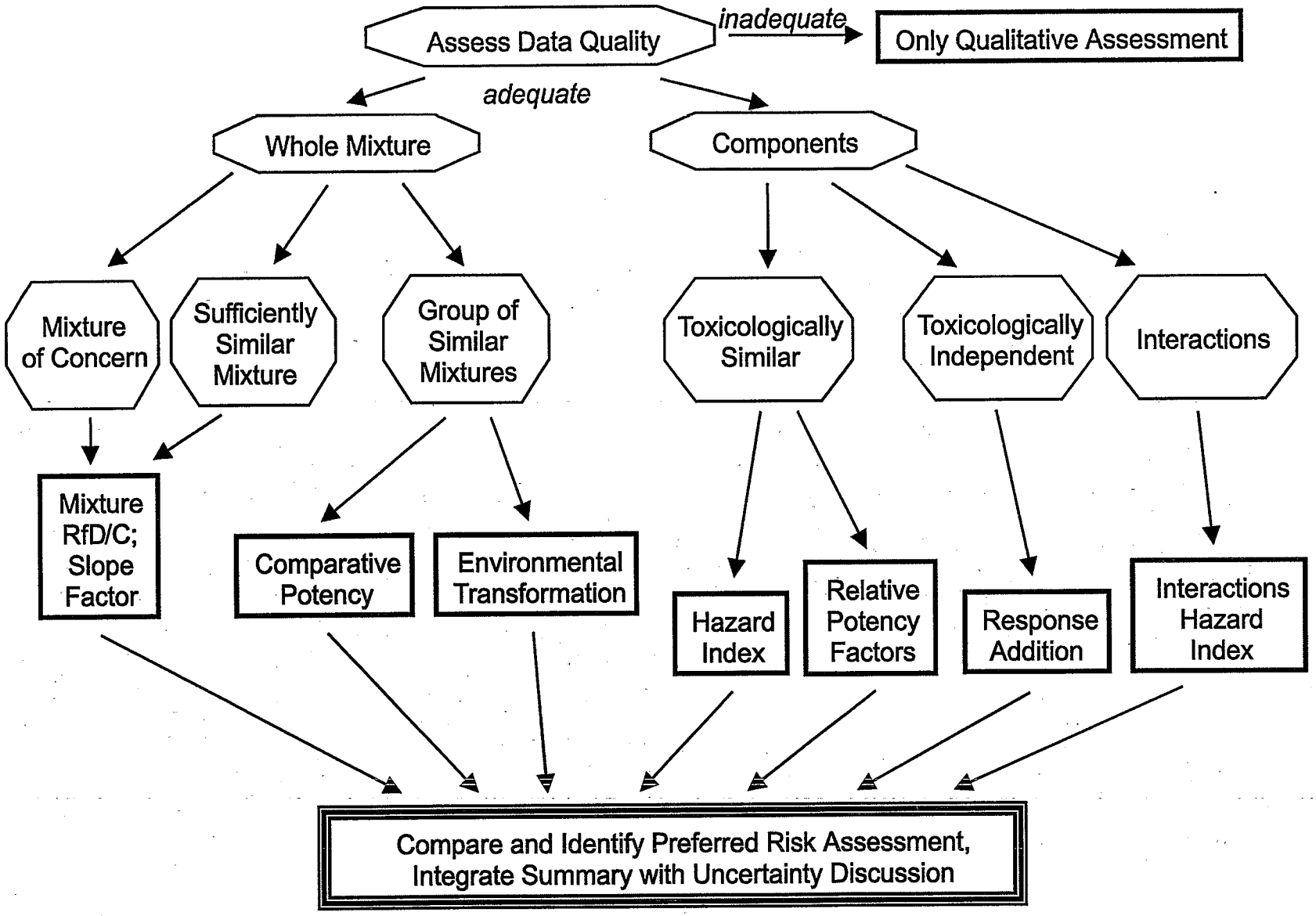


Figure 2-1. The different types of mixtures assessments based on the availability and quality of the data. All possible assessment paths should be performed.

- Data Requirements: details the types and amount of data that are needed to carry out the procedure.
- Section(s): refers the user to sections of this document that provide greater detail on the approach.
- References: cites reports or publications in which the approach has been applied in practice or indicates that this is a new procedure.
- Strategy of Method: provides concise directions on how the calculations are performed.
- Ease of Use: gives a sense of how much effort, expertise, and data are required in order to apply the approach.
- Assumptions: lists the toxicologic or statistical assumptions that are inherently made when the data are treated by applying the approach; the user can then decide if the approach is appropriate for the available data.
- Limitations: suggests problems the user may encounter relative to data gaps or quality deficiencies, and statistical modeling requirements or goodness-of-fit issues.
- Uncertainties: indicates unknown elements of the analysis that should be considered and characterized in the presentation of the risk assessment (e.g., data are not available, mode of action is unknown, scientific judgments are made, exposures are not well characterized, extrapolations are made, etc.).

Following an assessment of data quality, the first major distinction addressed in Figure 2-1 is whether the type of available data is whole mixture data or mixture component information. This distinction points the risk assessor toward methods that are available for these specific types of data. Methods available for whole mixtures then depend on whether there is information directly available on the mixture of concern or only on sufficiently similar mixtures or groups of similar mixtures. Methods available for component data then depend on whether there are interactions data available or whether the components act with a similar mode of action or are toxicologically independent. In these cases, the outcome is a quantitative assessment with a complete risk characterization and uncertainty discussion 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. In addition, there will often be mixtures for which there exist both whole-mixture and component data, so that the choice of method will not be clear (for example, both epidemiologic data and component toxicity data exist for evaluation of health effects from exposure to chlorinated drinking water). Furthermore, the true toxicologic mechanism of action (see Section 2.2.3) is rarely known for a given mixture or even for most of its components; thus the judgments that are made of toxicologic similar action or independence of action, for example, will be uncertain. It is recommended, therefore, that the risk assessor implement several of the approaches that are practical and evaluate the range of health risk estimates that are produced.

2.2.3. Key Concepts

There are several concepts that must be understood in order to evaluate a chemical mixture (see Appendix B). The first is the role of toxicologic similarity which, in this document, is considered along a continuum of information. The term mode of action is defined as a series of key events and processes starting with interaction of an agent with a cell, and proceeding through operational and anatomical changes causing disease formation. "Mode" of action is contrasted with "mechanism" of action, which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action. The specific term *toxicologic similarity* represents a general knowledge about the action of a chemical or a mixture and can be expressed in broad terms such as at the target organ level in the body (e.g., enzyme changes in the liver). In this document, assumptions about toxicologic similarity are made in order to choose among risk assessment methods. In general, we assume a similar *mode of action* across mixtures or mixture components and, in some cases, this requirement may be relaxed to require that these chemicals act only on the *same target organ.*

The second key concept in understanding mixtures risk assessment is the assumption of similarity or, in contrast, independence of action. The term *sufficiently similar mixture* refers to a mixture that is very close in composition to the mixture of concern, such that differences in their components and their proportions are small; the risk assessor can then use the data from the sufficiently similar mixture to make a risk estimate about the mixture of concern. The term *similar components* refers to the single chemicals within a mixture that act by the same mode of action and may have comparable dose-response curves; the risk assessor can then apply a component-based approach that uses these characteristics to form the basis of the risk assessment. The term *group of similar mixtures* refers to chemically related classes of mixtures that act by a similar mode of action, have closely related chemical structures, and occur together routinely in environmental samples, usually because they are generated by the same commercial process; the risk assessor can use what is known about the shifts in chemical structure and relative potency of the components to perform a risk assessment. Finally, the term *independence of action* is defined as mixture components that cause different kinds of toxicity, or effects in different target organs; the risk assessor may then combine the probabilities of toxic effects for the individual components.

Another key concept for this document is the understanding of language referring to toxicologic interactions, which is defined here as any toxic responses that are greater than or less than what is observed under an assumption of *additivity*. The term *additivity* is used when the effect of the combination of chemicals can be estimated directly from the sum of the scaled exposure levels (dose addition) or of the responses (response addition) of the individual components. There are a myriad of terms (see Appendix B, Table B-2) that represent various

kinds of interaction effects (e.g., inhibition, antagonism, masking). The most common and general of these refer to effects that are greater than additive (i.e., synergistic) or less than additive (i.e., antagonistic).

2.2.4. Qualitative Assessments

In Figure 2-1, an evaluation of the data may lead the user to decide that only a qualitative analysis should be performed. This generally occurs in cases where data quality is poor, there are inadequate quantitative data available, data on a similar mixture cannot be classified as "sufficiently similar" to the mixture of concern, exposures cannot be characterized with confidence, or method-specific assumptions about the toxicologic action of the mixture or of its components cannot be met. When this occurs, the risk assessor can still do a qualitative assessment that characterizes the potential human health impacts from exposure to that mixture. Such a risk characterization should discuss each element of the risk assessment paradigm, including available information on the mixture itself, on its components, and on potential interactions among the components. Any information on fate and transport of the mixture that would affect its final composition at the time of exposure should be noted.

2.2.5. 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. In many cases, information gaps will be too substantial to allow use of defaults, so that only a qualitative risk assessment can be performed. Nonetheless, for some restricted situations, default values and methods can be recommended. This section outlines the philosophy underlying their choice.

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 (Sections 2.6.2, 4.1, and 4.5) is recommended. For the component chemicals that show similar toxicity, dose addition (Sections 2.6.1, 4.1, 4.2, and 4.4) is recommended. Under dose addition, the general procedure is to scale the doses of the components by their relative potency and add the scaled doses together; the mixture response is then estimated for the combined dose. Under response addition, the general procedure is to first

determine the risks per the exposure for the individual components; the mixture 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. Finally, if interactions data are available, the default recommendation is that they be incorporated into the risk assessment by using the interaction-based Hazard Index (HI) (Sections 2.6.3, 4.1, and 4.3).

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 may be 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 EPA is the HI, where HI < 1 indicates a mixture exposure of no significant concern (U.S. EPA, 1989a). 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 dose-response curves of the components have congruent or similar shape (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 HI 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.

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 usually approximated by 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, 1989a). 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 that the dose-response curves have similar shape. It assumes that the components of the mixture are functionally independent of one another at low exposure levels (Mumtaz and Hertzberg, 1993), so that the risks may be added together (see Section 4.5 for details on interpretation and calculation). Because response addition does not

require a similar mode of action across the chemicals in the mixture, it allows for combining risks across chemicals even if they have different types of endpoints. An example is the combined risk of any kind of reproductive toxicity for a set of chemicals with different modes of action.

2.3. DATA QUALITY ASSESSMENT

The first consideration in Figure 2-1 is the assessment of data quality relative to its relevancy, completeness, quantitative nature, and certainty in three areas: exposure information, health effects information, and information on interactions. Table 2-1 presents a classification scheme for assessing the quality and nature of the available mixtures data. Consideration of the factors presented in Table 2-1 can be used to guide the risk assessor through Figure 2-1. This evaluation can assist the decision of whether to quantify the risk (the first step in Figure 2-1), and can be included in a discussion of overall quality of the risk assessment. Usually a classification of "FAIR" or better is required for quantitative risk assessment. 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 for quantification, with good data available for both the exposure and toxicity aspects of 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 toxicity value for the mixture, such as a Reference Dose (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 "FAIR" information on each of exposure, health effects, and interactions, 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 on the components in the assessment. Under these conditions, Figure 2-1 indicates that an interaction-based HI approach would be appropriate. It is the purview of the risk assessor to decide at what point the validity of the risk assessment is compromised by the data quality to such a degree that only a qualitative assessment should be performed.

2.3.1. Quality of Exposure Information

Exposure information ideally includes all data needed to characterize the human exposure to the mixture of concern from the point of environmental release to the point of human intake. There are several details needed to quantify exposure to chemical mixtures; these include:

Table 2-1. Classification scheme for the quality of available mixtures data[a]	
Exposure Information[b]	
GOOD –	Monitoring information either alone or in combination with modeling information is sufficient to accurately characterize human exposure to the mixture or its components.
–	Modeling information is sufficient to reasonably characterize human exposure to the mixture or its components.
FAIR –	Exposure estimates for some components are lacking, uncertain, or variable. Information on health effects or environmental chemistry suggests that this limitation is not likely to substantially affect the risk assessment.
–	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 –	The available exposure information is insufficient for conducting a risk assessment.
Health Effects Information	
GOOD –	Full health effects data are available and relatively minor extrapolation is required.
–	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.
FAIR –	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.
–	Certain important health effects data are lacking and extensive extrapolations are required for route or duration of exposure or for species differences.
POOR –	A lack of health effects information on the mixture and its components in the mixture precludes a quantitative risk assessment.
Information on Interactions	
GOOD –	Assessment is based on toxicologic data on the mixture of concern.
–	Assessment is based on data on a sufficiently similar mixture.
FAIR –	Quantitative interactions of all components are well characterized.
–	The assumption of additivity is justified based on the nature of the health effects and on the number of component compounds.
POOR –	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.

- Concentration of the chemical mixture in the medium/media of concern at the point(s) of human contact

- The duration and frequency of exposure should be developed from repeated measurements or validated models of environmental fate in media to which individuals are exposed, as well as human activity pattern data. The media concentrations should be determined at the points of human exposure. If the exposure data are limited, the analyst should address the degree to which the data

represent the environmental chemical mixture over space and time. Environmental transformation of the mixture over time is a key concern.

- Analytic chemistry

  The analyst should consider both the accuracy and reliability of the measurement techniques and determine if all of the components have been identified (i.e., are there unidentified components of the mixture?). The analyst should also determine if the key environmental reactions have been identified and reaction rates measured (e.g., environmental half-life) that govern the fate of the mixture. If components of the environmental mixture have not been detected analytically, the analyst should describe if and how they were included in the assessment (e.g., the compounds were assumed to be present at one-half the detection limit).

- Uptake from the environment

  The analyst should examine the bioavailability of the mixture for the medium and route of concern. The ideal data set would be derived from well-conducted studies that measure either the entire mixture or all the components in the pertinent exposure media and over the timeframe of concern. (The ideal data may be derived from accurate analytic measurements at points of human contact or from validated environmental fate models.) The magnitude of the human exposure would be measured or modeled on the basis of human activity patterns. Finally, the bioavailability of the mixture or the components would be known. Unfortunately, a complete data set is rarely available. The analyst should identify (and perhaps quantify) uncertainty based on imperfect analytic methods (e.g., some constituents may not be characterized by the analytic technique that represents the current state of the science), extrapolations between concentrations at measurement points and points of human exposure, incompletely understood transformation reactions to the mixture in the environment, and bioavailability. Each of these uncertainties in the risk assessment should be discussed and accounted for in the final risk characterization.

2.3.2. Quality of Health Effects Information

Health effects information includes both hazard identification and dose-response data on the complex mixture, a similar mixture, or the components of the mixture. The best data would be human epidemiologic or human clinical data directly on the complex mixture for which the health effects of concern are causally linked to the mixture exposure and a dose-response relationship can be established for the exposure route of interest. Unfortunately, such high-quality direct information is rarely available, so the risk assessor usually performs one or more extrapolations. Examples of such extrapolations include using animal data to project potential human health effects, using inhalation data to predict risks from oral exposure, using component data to estimate risks for the complex mixture, and using data from short-term human clinical

studies or subchronic animal bioassays to project human health risks from chronic exposure. Each of these extrapolations introduces uncertainty into the risk assessment that should be discussed and accounted for in the final risk characterization.

2.3.3. Quality of Interactions Information

Interactions information includes any data indicating that the toxicologic action of the complex mixture is greater than or less than what might be expected from exposure to a colleciton of individual components of the mixture. Thus, human or animal data directly on the whole mixture implicitly provides interactions information for use in risk assessment. However, since such data are rarely available, the risk assessor must often rely on component information, the vast majority of which is laboratory toxicity data on binary combinations of chemicals (Teuschler and Hertzberg, 1995). The quality of interactions data, whether it be data on the complex mixture, a sufficiently similar mixture, or simple combinations of the components, can be judged according to the strength of evidence for three criteria. First, there should be adequate toxicity data that not only provide information on dose response, but also on the mechanism of action for the mixture. Second, interactions data should be for the same route of exposure as the mixture of concern. Furthermore, when data on several different component mixtures are evaluated, these data should be from comparable studies, such as the same species, same endpoint of concern, similar laboratory conditions, or comparable study duration. Finally, observed interactions data that are usable for risk assessment purposes should be toxicologically significant (i.e., show definite adverse effects). The strength of the evidence for each of these criteria should be discussed and accounted for in the final risk characterization.

2.4. CHEMICAL MIXTURE EXPOSURE ASSESSMENT ISSUES

While this guidance document is intended to serve risk assessors primarily by informing them of dose-response and risk characterization methods associated with exposures to chemical mixtures, the purpose of this section is to highlight additional exposure issues of a *general* nature that should be considered when developing a risk assessment for chemical mixtures. The issues presented in this section should be considered in addition to those normally followed in an exposure assessment. The Agency's primary guidance in this area is the Exposure Assessment Guidelines (U.S. EPA, 1992); however, that document primarily focuses on issues pertaining to single-chemical exposures. Other, more specific exposure assessment issues involving multiple chemicals will be discussed by the Agency more comprehensively in separate future efforts (e.g., the EPA's Risk Assessment Forum is developing a cumulative risk assessment framework as this guidance goes to press). While there are other important issues related to exposures to chemical

mixtures, three critical areas will be discussed briefly here: environmental fate, temporal patterns of exposure, and routes of exposure.

The wide diversity in mixture compositions and site characteristics precludes any recommendation for a single approach for site-specific modification of the mixture assessment. Through examples, some steps that should be considered can be articulated. The example in Section 3.4 demonstrates some of the considerations that should 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. Clearly, the analyst should report the significance of any assumptions utilized as well as the potential uncertainty and variability associated with the exposure modifications developed for the risk assessment.

2.4.1. Environmental Fate and Transport

The composition and quantity of a mixture of chemicals may change after release into the environment. The environmental fate of chemical mixtures released into the environment can be conceptualized as being composed of three *interrelated* components: (1) transport through an individual compartment (e.g., atmospheric dispersion); (2) transfer between environmental compartments (i.e., partitioning); and (3) transformation mediated by biological, chemical, or physical processes (e.g., weathering) (Crawford-Brown, 1997, Chapter 2). Even though the environmental processes that occur within these three components of environmental fate are not unique to chemical mixtures, the analyst should assess compositional and quantitative changes that may occur to the chemical mixture of interest in the environment (particularly with respect to the time from release to exposure), and the impact these will have on exposure and toxicity.

This is particularly important when considering the appropriateness or relevance of an analytic measurement of quantity or composition of a chemical mixture; the analyst needs to consider the possible changes to the mixture between the time the measurement was conducted and the time over which exposures are expected to occur. These environmentally mediated changes are also important when comparison is made in the assessment to the dose response exhibited by either a sufficiently similar whole mixture (e.g., comparison of the dose response of the commercial mixture that has been toxicologically tested to that of the environmental mixture) or mixture components. The concept of *sufficient similarity* is not discussed in the 1986 mixtures guidelines (U.S. EPA, 1986, 1987) (Appendix A). Common sense dictates that *sufficient similarity* entails the assumption that the toxicologic consequences of exposure to the two mixtures (i.e., the mixture of concern and the mixture on which data are available) will be identical or at least indistinguishable from one another. In practice, some degree of chemical similarity or at least an understanding of how chemical differences between the mixtures affect

toxicological activity is required. The acceptability of a surrogate, given the degree of accuracy desired in the risk assessment, should be identified in the analysis.

When the effects of such environmental processes cannot be directly measured or modeled on the mixture of interest, 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. The degree to which environmental fate alters the exposure or the dose response changes a basic assumption of risk assessment of chemical mixtures, that of sufficient similarity. Under some circumstances, sufficiency of similarity may be gauged by the gradient of costs (monetary or environmental) of misjudging similarity, although such analyses will not be discussed here.

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 should 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.

2.4.1.1. *Transport Through an Environmental Compartment*

Transport of a chemical mixture through the environmental compartments of air, soil, and water will depend upon the physical and chemical properties of the individual components or the unique properties of the chemical mixture (e.g., nonaqueous-phase liquids [NAPLs]) and the environmental medium. There are a number of examples of changes in composition or quantity of a chemical mixture as a result of environmental fate. The changes in the quantities and concentrations of chemical disinfectant by-products (occuring in chemically disinfected drinking water over time) during transport through the drinking water distribution system provide an example of the changes that can occur to a mixture during transport through an environmental compartment.

2.4.1.2. *Intercompartmental Transfer Between Environmental Compartments*

All components of a chemical mixture may not be transferred between environmental compartments at the same rate. Once released to the environment, a mixture of chemicals may be partitioned on the basis of the physical/chemical properties of each component of the mixture and the condition of the microenvironment into which the components are partitioned.

Selective movement of components can occur primarily during transport between soil, air, or water environments. For example, volatilization from the soil surface compartment to the atmospheric compartment could be important initially for the more volatile compounds in the mixture. Volatilization from dry soil surfaces is dependent on both the vapor pressure (more volatile compounds will volatilize more readily) and the ability of a compound to adsorb to soil. Volatilization from moist soil surfaces is driven by the Henry's Law constant at steady state (volatilization increases with a larger Henry's Law constant) and, as with dry soil surfaces, the ability of a compound to adsorb to the soil. Because the Henry's Law constant is defined as the ratio of a compound in air to that in water, compounds with either a high vapor pressure or compounds that have a low vapor pressure together with a low water solubility may volatilize from both moist soil and water surfaces. The rate at which a compound can volatilize from the soil surface may be attenuated if that compound is also able to adsorb strongly to soil particles. Compounds that adsorb strongly to the soil may also be physically entrained in the air as dust or moved to aquatic environments via sediment runoff. Compounds that do not adsorb strongly to the soil may leach readily through the soil column to groundwater systems if processes such as volatilization and biodegradation do not occur rapidly enough. (There are exceptions, such as where some vapor-phase pollutants in stack emissions adsorb to particulates.) The extent of soil adsorption is generally based on the organic content of the soil, although some compounds (those with a positive charge) can also adsorb to clays. A soil adsorption coefficient is defined in terms of the soil organic carbon and can be used to estimate the ability of a particular compound to leach into the soil column. The more volatile components of a chemical mixture in soil may volatilize over a several-year period and no longer be present. A risk assessment based only on the original mixture composition could then overestimate the long-term risk if the volatile chemicals were the primary toxicants. Adjustments based on other factors such as exponential decay models calibrated for the soil composition being assessed might improve the risk estimate.

The analyst should also consider differential transfer of chemicals comprising a mixture between abiotic and biotic compartments and between two different biotic compartments. For example, certain dioxin congeners released from the stacks of combustion sources appear to be selectively taken up and retained in plant tissues (Lorber et al., 1996; 1998). The relative proportions of dioxin congeners in the mixture to which humans and grazing animals are exposed through the consumption of these contaminated plants vary considerably from the original congener mixture released to the environment. The proportions of dioxin congeners in human exposures that result from consumption of the tissues of the grazing animals (e.g., beef cattle) will differ from the proportions released from the stack as well as those in the contaminated plants.

2.4.1.3. *Transformation of a Chemical Mixture or Individual Compound Into Degradation Products*

In the environment, chemical mixtures may arise or change as a result of transformation. If the various compound/s are susceptible to degradation via photolysis, hydrolysis, or biodegradation (both aerobic and anaerobic), both alteration of the profile of the original compounds in the mixture and changes in the quantity of the mixture present are possible. The processes acting to change the profile of a mixture may be affected by the point of release of the mixture (i.e., the profile from a mixture directly released to a lake may be different from that from the same mixture following long-range atmospheric transport). Transformation reactions that may differentially affect mixtures components in air, soil, and water are presented below, followed by an example using the transformation of toxaphene.

- Atmosphere: Compounds can be transformed by direct photolysis, if the compound is able to absorb light in the visible region of the spectrum, and/or by reaction with reactive photochemically generated hydroxyl radicals, nitrate radicals, and ozone (Atkinson, 1994). Reaction with hydroxyl radicals is expected to be the major degradation process in the troposphere for most molecules, and the rate of this reaction depends primarily on the chemical structure (Atkinson, 1994). Unsaturated compounds also are expected to react quickly with nitrate radicals and ozone.

- Soil: Compounds can be transformed through aerobic and anaerobic biodegradation at the soil surface. Aerobic biodegradation is controlled by concentrations of oxygen and nutrients; compounds susceptible to anaerobic biodegradation may be transformed in anaerobic microsites, which may be found within the soil column and when the soil is flooded.

- Water: Susceptible compounds may be transformed through hydrolysis (e.g., structures such as amides, alkyl halides, carbamates, and phosphoric acid esters [Lyman et al., 1990] are particularly vulnerable), direct photolysis at the water surface, and aerobic biodegradation.

The assessment of environmentally degraded or "weathered" toxaphene, previously the most heavily used pesticide in the United States, exemplifies the concerns of transformation as well as other environmental fate processes when developing a chemical mixtures risk assessment. Toxaphene is a formulation of multiple ingredients. The relative amounts of these components and their character change after toxaphene is released to the environment and the original components of the mixture are exposed to differential partitioning and transformation processes in air, water, and soil environments (U.S. EPA, 1997b).

- Toxaphene congeners are generally biologically degraded under anaerobic conditions through reductive dechlorination. Anaerobic degradation rates in soils and sediments are expected to be determined largely by qualities of the original component molecules and the environment's potential to interact and change the molecules' structure (Fingerling et al., 1996; Smith and Willis, 1978). The stability of reaction products, whether in soil or sediment, seems to depend on the position of the various chlorine atoms.
- Under aerobic conditions toxaphene degrades slowly, if at all (Parr and Smith, 1976; Bidleman et al., 1981; Mirsatari et al., 1987; Nash and Woolson, 1967).
- In general, the lower chlorinated toxaphene congeners are more easily vaporized than are the higher chlorinated congeners (Seiber et al. [1979] showed soil surface enrichment of the less volatile, more chlorinated compounds through GC analysis); however, both can be atmospherically transported.
- Toxaphene, particularily the more volatile components, may be transported far from the initial source by long-range atmospheric transport processes.
- Once deposited in water, the higher chlorinated congeners can bioaccumulate in the food chain because of their lipophilicity.

The composition of "weathered" toxaphene samples may be different, depending on the environmental processes to which the original agent was exposed. For example, toxaphene extracted from an anaerobic soil does not resemble that from an aerobic soil, and toxaphene detected in an air sample from the Arctic does not resemble the toxaphene residue obtained from the blubber of an Arctic seal. Site-specific consideration of the partitioning and transformation processes is needed for different environments. The resulting changes in chemical composition of the original mixture over time will affect the toxicity of the mixture.

For another example, when the primary change to a mixture is believed to be the degree of halogenation or other substitution, some adjustment of the estimated exposure or toxic potency may be possible. One example (discussed in Section 3.4) concerns combinations of PCBs, for which EPA has developed specific methodology to alter the toxic potency on the basis of site-specific environmental factors.

2.4.2. Importance of the Exposure Sequence for Multiple Chemicals

The order in which chemical exposures occur and the time between exposures to different chemical agents may affect the nature of the response to the chemical insult. For example, the sequence or pattern of exposure is important for compounds that have been described as initiators and those described as promoters of carcinogenicity. There is evidence to suggest that exposure to certain compounds results in an irreversible change in the affected cells and progeny (the cell is said to be initiated). When the initial exposure is followed by repeated doses of a second chemical agent (i.e., the promoter), tumors occur. In the absence of either the initiator or the

promoter, or if the order is reversed, tumors do not occur. An example of an initiator-promoter sequence is the application of a PAH (initiator) (e.g., benzo[b]fluoranthene) followed by repeated applications of 12-o-tetradecanoyl phorbol-13-acetate (TPA) to the skin of shaved mice (Amin et al., 1985).

2.4.3. Routes of Exposure

In environmental health risk assessments, analysts typically consider three routes of human exposure: oral, dermal, and inhalation. Differences in the properties of the cells that line the surfaces of the gastrointestinal tract, the skin, and the air pathways and lungs may result in different intake patterns of chemical mixture components depending on the route of exposure. Additionally, chemicals in a mixture may partition to contact media differently, resulting in different potential routes of exposure (see Section 2.4.1). In chemical mixtures risk assessment, the issue becomes how and when to combine routes. EPA is still developing approaches for this. EPA (1998c) recommends that route-to-route conversion should be attempted only for dermal exposures at this time. Adequate inhalation-to-oral conversion methods for steady-state conditions have not yet been developed. A general outline of the oral-to-inhalation extrapolation process and a discussion of route-to-route extrapolation issues can be found in Gerrity and Henry (1990) and in EPA's Reference Concentration methodology document (U.S. EPA, 1994a). Until such methodology is developed, inhalation and oral risk characterization should be carried out separately. The assessor should note, however, that total risk from the mixture could be underestimated by not combining all routes of exposure, because the total exposure is not characterized and the chemical interactions may not be considered.

Multiple-route exposures can be combined in two different ways: summing the absorbed daily doses or summing the (external) oral equivalent daily doses. Both approaches require an estimate for the oral absorption fraction, but the latter is adopted here as it is simpler for consideration with standard toxicity comparison values based on ingestion (e.g., RfD).

A number of factors might contribute to differences in toxicologic effectiveness between oral and dermal exposures at equal dosages. The most obvious relates to differences in absorption rates between the two routes. Other potential contributing factors include differing sensitivity of absorption sites to damage and differences in toxicokinetics (i.e., distribution, metabolism, elimination) between exposure routes. Ideally, the conversion from dermal to equivalent oral dose would be based on experimentally derived values that characterize the relationship between the doses that produce a particular toxicity by each of the different routes. In practice, however, the conversion usually will be based on absorption factors because of a general absence of appropriate data.

2.4.4. Exposure Assessment Summary

This section summarizes a few important concepts related to chemical mixtures exposure assessment. Once a chemical mixture is released to the environment, its concentration and composition may change through partitioning into abiotic and biotic compartments and through transformation mediated by the environment and biota. The physical/chemical properties of each component of the mixture (or the properties of the mixture as a whole) and the condition of the microenvironment into which the components are partitioned may change the magnitude and the routes of human exposure. Partitioning and transformation of the mixture components will affect the routes of exposure. Ideally, chemical mixture exposures through different routes can be integrated through measurement data or a validated physiologically based pharmacokinetic (PBPK) model; at this time, approaches are still evolving, particularly for combining inhalation and oral exposures. The sequence of exposures to different chemical agents is clearly important for some responses. A number of other issues will be deferred for later discussion by the Agency; these include chemical mixtures with intrinsically unique properties (e.g., NAPLs), mass balance within chemical mixtures assessments, assessing risk of unidentified components of chemical mixtures, measurement issues, and component bioavailability.

2.5. DATA AVAILABLE ON WHOLE MIXTURES

Whenever possible, the preferred approach to the health risk evaluation of chemical mixtures is to perform the assessment using health effects and exposure data on the whole mixture. Such data include human epidemiologic, clinical, or occupational studies; animal studies on the complex mixture; or in vitro data on the complex mixture. Figure 2-1 shows that the whole-mixtures data can then be divided into subsets of data directly on the mixture of concern, data on a sufficiently similar mixture, or data on a group of similar mixtures. This guidance document discusses these situations and offers some examples of how to approach a whole-mixture health risk assessment.

2.5.1. Data Available on the Mixture of Concern

Exposure and toxicity data directly on the mixture of concern are most likely to be available for highly complex mixtures, such as coke oven emissions, which are generated in large quantities and associated with or suspected of causing adverse health effects. The evaluation of such a mixture requires scientific judgment regarding the stability of the mixture in the environment and the linkage of the observed human health effect to the mixture exposure. 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 2.5.3, 3.3).

2.5.1.1. *User Fact Sheet: Mixture of Concern RfD/C or Slope Factor*

The user of this guidance document can use Figure 2-1 to determine if data are available directly on the mixture of concern. Then a procedure is suggested for estimating either a cancer slope factor or a reference dose/concentration (RfD/C), as encapsulated in the following user-information fact sheet.

Approach:	Mixture of Concern RfD/C or Slope Factor
Type of Assessment:	Dose-Response Toxicity Value
Section(s):	3.1, 3.2
References:	Examples can be found on IRIS (U.S. EPA, 2000a).
Data Requirements:	Toxicity data are available on the mixture of concern. Examples of such data are human epidemiologic data from an occupational setting, human data from a clinical study, or animal toxicology data on the complex mixture.
Strategy of Method:	Estimate dose-response toxicity value directly from data on complex mixture of concern, using the same procedures as those used for single chemicals.
Ease of Use:	Calculations are simple.
Assumptions:	Composition of the test mixture is functionally the same as what is found in the environment. Test data are adequate to account for all sensitive endpoints.
Limitations:	Data are rarely available.
Uncertainties:	Scientific judgments of the chemical composition of the mixture; toxicologic relevance of the laboratory data to the environmental mixture.

2.5.2. Data Available on a Sufficiently Similar Mixture

If data are not available on the mixture of concern, the risk assessment may be based on data on a sufficiently similar mixture. A mixture is sufficiently similar to the mixture of concern when its components are not very different and are contained in about the same proportions as the mixture of concern. In addition, if information exists on differences in environmental fate, uptake and pharmacokinetics, bioavailability, or toxicologic effects for either of these mixtures or their components, it should be considered in the determination of sufficient similarity. If such data are available, an attempt should be made to determine if significant and systematic differences exist between the chemical mixtures. If no significant differences are noted, then a risk assessment may be performed using data on the sufficiently similar mixture as a surrogate for the mixture of concern.

2.5.2.1. *User Fact Sheet: Sufficiently Similar Mixture RfD/C or Slope Factor*

The user of this guidance document can use Figure 2-1 to determine that the data available are on a mixture that is sufficiently similar to the mixture of concern. Then a procedure is suggested for estimating either a cancer slope factor or a reference dose/concentration (RfD/C), as encapsulated in the following user-information fact sheet.

Approach:	Sufficiently Similar Mixture RfD/C or Slope Factor
Type of Assessment:	Dose-Response Toxicity Value
Section(s):	3.1, 3.2
References:	New procedure.
Data Requirements:	Toxicity data are available on a mixture that is judged as sufficiently similar to the mixture of concern in the environment. No data are available on the mixture of concern. Examples of such data are human epidemiologic data from an occupational setting, human data from a clinical study, or animal toxicology data on the complex mixture.
Strategy of Method:	Estimate dose-response toxicity value using data on the sufficiently similar mixture as a surrogate for data on the mixture of concern, using the same procedures as those used for single chemicals.
Ease of Use:	Calculations are simple.
Assumptions:	Composition of the sufficiently similar mixture is functionally the same as what is found in the environment. Test data are adequate to account for all sensitive endpoints. Similarity judgment across the mixtures must be made and supported.
Limitations:	Availability of data is limited.
Uncertainties:	Scientific judgments of sufficient similarity, chemical composition and stability of the two mixtures; toxicologic relevance of the laboratory data to the environmental mixture.

2.5.3. Data Available on a Group of Similar Mixtures

In some cases, data are available on a group of similar mixtures that are known to be generated by the same commercial process or emissions source but that vary slightly in composition depending on factors such as time since emission, environmental transformation, or geographic location of emission sources. Data are then available on several mixtures with approximately the same components but with slightly different component exposure levels, so that the likely range of compositional variation is covered. Thus, risk assessors can use toxicity and exposure data that exist on the group of similar mixtures and extrapolate in order to perform a risk assessment on the less well-studied or environmentally transformed mixtures that belong to that same group.

2.5.3.1. *User Fact Sheet: Comparative Potency*

The user of this guidance document can use Figure 2-1 to determine that the data available are on a group of similar mixtures. Then a procedure is suggested for using a comparative potency approach to estimating a cancer slope factor, as encapsulated in the following user-information fact sheet.

Approach:	Comparative Potency
Type of Assessment:	Dose-Response Toxicity Values for Cancer, Genetic Toxicity
Section(s):	3.1, 3.3
References:	Used for combustion mixtures (Lewtas, 1985, 1988; Nesnow, 1990).
Data Requirements:	Method requires short-term data on several similar mixtures including the mixture of concern, and at least one data point from a chronic in vivo study on one of these mixtures. Examples of such data are in vitro mutagenicity assays and chronic rodent bioassays.
Strategy of Method:	Estimate dose-response value using relationships across similar mixtures and similar assays to extrapolate to a value for the mixture of concern.
Ease of Use:	Calculations involve some statistical modeling and toxicologic judgment. Method is data intensive with short-term assay data required.
Assumptions:	Assumes the potency change for similar mixtures across assays is the same for all similar mixtures. Test data are adequate to account for all sensitive endpoints. Similarity judgment across the mixtures must be made and supported.
Limitations:	Availability of data is limited.
Uncertainties:	Scientific judgments of sufficient similarity relative to chemical composition and toxicologic activity of the mixtures.

2.5.3.2. *User Fact Sheet: Geographic Site-Specific Assessments*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on a group of similar mixtures. Then a procedure is suggested for estimating risk from exposure to the mixture by using a geographic site-specific assessment, as detailed in the following user-information fact sheet.

Approach:	Geographic Site-Specific Assessment
Type of Assessment:	Risk Characterization for Any Toxic Endpoint
Section(s):	3.1, 3.4
References:	Used for cancer assessment of PCBs (U.S. EPA, 1996c)
Data Requirements:	Method requires both toxicity and exposure data on the mixture's components.
Strategy of Method:	Toxicity data on the commercial mixture are used to estimate a range of toxicity values that are then adjusted for alterations in the mixture's composition because of environmental factors to produce a risk estimate for the total mixture.
Ease of Use:	Complicated to use. Data intensive.
Assumptions:	Requires the user to make assumptions about the fate and transport of groups of chemicals.
Limitations:	Some data restricted by similarity. Restricted to specific conditions. Limited by data quality.
Uncertainties:	Scientific judgment of fate and transport. Accuracy of exposure data.

2.6. DATA AVAILABLE ON MIXTURE COMPONENTS

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. The primary criterion for choosing between dose addition and response addition is the toxicologic similarity among the chemicals in the mixture. This decision should be based on information about the toxicologic and physiologic processes involved, the single-chemical dose-response relationships, and the type of response data available. The risk assessment using component data should then begin with selection of the most appropriate concept for the chemicals in the mixture.

2.6.1. Toxicologic Similarity and Dose Addition

In the simplest terms, chemicals can be considered as dose additive if each chemical can be thought of as a concentration or dilution of every other chemical in the mixture. 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 description of dose addition requires a constant proportionality between the effectiveness of the two chemicals. Three component methods that are based on dose addition are discussed in this document: the HI, the Relative Potency Factor (RPF) method, and the Toxicity Equivalence Factor method, which is a special case of the RPF 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.

2.6.1.1. *User Fact Sheet: Hazard Index*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on the components of the mixture of concern and that there is evidence of toxicologic similarity of the components. Then a procedure is suggested for estimating a Hazard Index, an indication of risk from exposure to the mixture, as encapsulated in the following user-information fact sheet.

Approach:	Hazard Index
Type of Assessment:	Risk Characterization for Any Toxic Endpoint
Section(s):	4.1, 4.2
References:	Used in Superfund site assessments (U.S. EPA, 1989a).
Data Requirements:	Method requires both toxicity and exposure data on the mixture's components. Good dose-response data are needed, such as what is available on IRIS (U.S. EPA, 2000a).
Strategy of Method:	Scale individual component exposure concentrations by a measure of relative potency (typically, divide by a Reference Dose/Concentration [RfD/C]) for components with a similar mechanism-of-action. Add scaled concentrations to get an indicator of risk from exposure to the mixture of concern.
Ease of Use:	Easy to calculate.
Assumptions:	Applies dose addition, which carries with it assumptions of same mode of action and similarly shaped dose-response curves across the components. The "common mode-of-action" assumption can be met by using a surrogate of same target organ.
Limitations:	Exposure data should be at relatively low levels (near no-adverse-effect levels) at which interaction effects are not expected. RfD/C values across components vary in their uncertainty, so other measures of potency may be more appropriate.
Uncertainties:	Similarity of mechanism-of-action. Accuracy of exposure data.

2.6.1.2. *User Fact Sheet: Relative Potency Factors*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on the components of the mixture of concern and that there is evidence of toxicologic similarity of the components. Then a procedure is suggested for estimating risk from exposure to the mixture by using Relative Potency Factors, as encapsulated in the following user-information fact sheet.

Approach:	Relative Potency Factors
Type of Assessment:	Dose-Response Assessment for Any Toxic Endpoint
Section(s):	4.1, 4.4
References:	New Procedure
Data Requirements:	Method requires both toxicity and exposure data on the mixture's components. Toxicity data are missing for some components.
Strategy of Method:	Scale component exposure concentrations relative to potency of an index chemical (typically the best-studied component) following expert committee consensus. Add scaled concentrations. Use dose-response curve of index chemical to generate response estimate for sum of scaled concentrations.
Ease of Use:	Complicated to use. Requires some statistical modeling and judgment of relative potency factors.
Assumptions:	Based on dose addition which carries with it assumptions of same mode of action and similarly shaped dose-response curves across the components. The "common mode-of-action" assumption can be met using a surrogate of toxicologic similarity, but for specific conditions (endpoint, route, duration).
Limitations:	Limited by data quality and similarity. May not have data from all routes of exposure of interest. Same mode-of-action across components may not be

2.6.2. Independence and Response Addition

Response addition may apply when components act on different systems or produce effects that do not influence each other. Under response addition, the chemicals in the mixture 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. Mathematically, response addition can be described by the statistical law of independent events, with "response" measured by the percentage of exposed animals that show toxicity or the proportion of the population responding. Response addition is particularly useful when the effects of concern are thought to be present at low dose levels for each of the component chemicals, even though it is highly unlikely the effects are capable of being observed at these low levels in the environment. When interaction data are available on any of the components in the mixture, the risk assessor may provide a qualitative discussion of the likely effect of these data on the outcome of the mixture risk assessment under response addition (see Sections 2.2.4, 4.5.4).

2.6.2.1. *User Fact Sheet: Response Addition*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on the components of the mixture of concern and that there is evidence of toxicologic independence of action. Then a procedure is suggested for estimating risk from exposure to the mixture by using Response Addition, as encapsulated in the following user information fact sheet.

Approach:	Response Addition
Type of Assessment:	Risk Characterization for Any Toxic Endpoint
Section(s):	4.1, 4.5
References:	Used extensively for cancer. Used in Superfund site assessments (U.S. EPA, 1989a).
Data Requirements:	Method requires both toxicity data (measured in percent responding) and exposure data on the mixture's components. Good dose-response data are needed, such as what is available on IRIS (U.S. EPA, 2000a).
Strategy of Method:	Risk of an effect is estimated for each component using its dose-response curve at the component's exposure concentration. Component risks are added, using the independence formula, to yield a risk estimate for the total mixture for the specific exposure.
Ease of Use:	Easy to calculate.
Assumptions:	Assumes toxicologic independence of action. Assumes interactions are not significant at low exposures.
Limitations:	Limited to low exposure concentrations. Slight overestimate of mixture's upper bound on risk when adding individual component upper bound estimates. Restricted to independence of action.
Uncertainties:	Independence of action. Accuracy of exposure data. Individual risk estimates may vary in quality.

2.6.3. Interactions Data

Toxicologic interactions are operationally defined by the existence of data showing significant deviations from a "no interaction" prediction; that is, the response is different from what would be expected under an assumption of additivity (e.g., dose-additive, response-additive). Types of interactions among mixture components that can affect toxicologic response to the whole mixture include chemical-to-chemical, toxicokinetic, and toxicodynamic interactions (see Table B-2 and Appendix C). The impact of these constituent interactions on toxicologic response can be less than additive (e.g., antagonistic) or greater than additive (e.g., synergistic). The component-based method discussed in this document that incorporates interactions information is the interaction-based HI.

2.6.3.1. *User Fact Sheet: Interaction-Based Hazard Index*

The user of this guidance document can follow Figure 2-1 to determine that the data available are on the components of the mixture of concern and that interactions data are available. Then a procedure is suggested for estimating risk from exposure to the mixture by incorporating information on binary combinations of the components using an interaction-based hazard index, as encapsulated in the following user information fact sheet.

Approach:	Interaction-Based Hazard Index
Type of Assessment:	Risk Characterization for Any Toxic Endpoint
Section(s):	4.1, 4.3
References:	New procedure (Hertzberg et al., 1999).
Data Requirements:	Method requires both toxicity and exposure data on the mixture's components, and interactions data on at least one pair of components.
Strategy of Method:	Scale component exposure concentrations by a measure of relative potency (typically, divide by a reference dose/concentration [RfD/C]) for components with a similar mechanism-of-action. Modify this term with data on binary interactions. Add scaled/modified concentrations to provide an indicator of risk from exposure to the mixture of concern.
Ease of Use:	Complicated to use.
Assumptions:	Assumes binary interactions are the most important. Assumes interaction magnitude is not dose dependent, but depends on component proportions.
Limitations:	Limited interactions data are available. Model with relative proportions is untested. Interaction magnitude is often a default because of lack of measurement data.
Uncertainties:	Binary interactions used to represent the interactions for the whole mixture. Accuracy of exposure data. Accuracy of default for interaction magnitude.

2.7. FUTURE DIRECTIONS

2.7.1. 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 (Cassee et al., 1998). The characteristics to include in a mixture prioritization scheme should parallel those often cited for single chemicals: target

those mixtures posing the highest public health risk. The supporting data could include annual emissions of mixtures, frequency of occurrence of mixtures in the environment, identity of mixtures containing highly toxic chemicals, or documented health problems in populations exposed to identified mixtures. Because most interaction data are on chemical pairs, one approach would include the frequency of occurrence of chemical pairs in the media associated with the exposure scenario to be regulated. The prioritization should also consider the availability of interaction data. For high-priority mixtures lacking such data, other assessment methods may be needed. The various regulatory program areas, such as Superfund waste sites, ambient air, and drinking water, pose substantially different kinds of mixtures and exposure conditions, so that a priority list for one program may not be appropriate for a different regulatory program.

Once a few mixtures posing the highest concern have been identified, researchers 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, short-term toxicity assays, 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 to the scenario of concern. In addition to the issues being addressed by extrapolation methods for single chemicals (e.g., cross-species, cross-route), mixtures issues also include interactions and changes in composition. Interactions issues include the commonly observed toxicologic interactions that influence pharmacokinetics, as well as the less-studied areas of physiological interactions between affected tissues or organs, and the biochemical and physical interactions affecting degradation and transport of mixtures in environmental media. Because of the wide variety of mixture exposures, all relevant information should be tapped to improve the understanding of the basic biological and chemical processes. For example, to improve dose-response extrapolation, toxicology experiments, epidemiology and occupational studies, and mathematical model development should be pursued simultaneously.

Mixtures research should be efficient. The complexity of the issues is beyond the reach of any single agency. Sharing of resources and information within different sectors of EPA as well as with other agencies is essential. Several such efforts are underway. The Integral Search System (Arcos et al., 1988) and the Mixtox database (Marnicio et al., 1991) are two EPA collections of bibliographic summaries of interaction studies that are available to the public. Additional databases should be developed, perhaps jointly with the public, on mechanisms and modes of toxicologic interaction and on mathematical models of biological processes influencing

the interactions. The National Institute for Occupational Safety and Health (NIOSH) has a Mixed Exposures research program whose advisory committee includes representatives from EPA, other federal agencies, and research institutions. EPA, NIOSH, and the Agency for Toxic Substances Disease Registry (ATSDR) have organized the Mixed Exposures Research Group (MERG), composed of almost 20 federal and state agencies, to share regulatory approaches. MERG seeks to facilitate interagency communication and jointly sponsored research projects on mixtures. Additional cooperative efforts should be pursued with the public and foreign agencies.

Mixture risk assessment methods should ideally be developed in conjunction with those laboratory and field studies that are needed for implementation as well as validation. Otherwise, the methods become conceptual models that cannot feasibly be applied, or decision tools whose accuracy cannot be tested. One example concerns interaction studies, such as those detailed in the EPA's Mixtox database (Marnicio et al., 1991; U.S. EPA, 1990) of in vivo toxicologic interaction studies. In the Mixtox database, 95% of the studies involve only pairs of chemicals (Teuschler and Hertzberg, 1995). Consequently, the interaction-based Hazard Index (Section 4.3) was developed for pairwise interactions to allow use of available data. Interaction studies are in progress by research groups in EPA's National Center for Environmental Assessment (NCEA) and National Health and Environmental Effects Research Laboratory (NHEERL) to provide the toxicity data and data analysis methods for validation of the index.

The information required for evaluation of the extrapolation methods in this document is generally not yet available. The number of pairs studied for interactions 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 190 pairs, but over a million possible combinations (pairs, triples, etc.). 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 mixture risk discussed in this document are then likely to be adopted based on scientific plausibility and on relatively few validation studies. The validation process is valuable, even when incomplete. As was found with the analysis of the consistency of pairwise interactions (Durkin et al., 1995), the evaluation of the mixture risk tools will likely spawn research questions that lead to new statistical, exposure, and toxicologic studies, and subsequently to better risk tools.

2.7.2. Research Suggestions for Improving Mixture Risk Assessment

Several research directions have been suggested during the development of this guidance document. Although specific projects have been identified related to dose-response assessment, the highest priority was the preparation of guidance on exposure assessment of mixtures. Some of the key concerns with exposure assessment are discussed in this document (Section 2.4). The

need is for specific procedures for measurement and modeling of exposures for various scenarios, along with the corresponding methods for characterizing the uncertainties. The Risk Assessment Forum created an advisory panel in 1999 to decide the scope and project requirements for a framework for cumulative risk assessment. A major component of that framework is the exposure assessment of mixtures. Some specific areas for exposure assessment that have been suggested during review of this guidance are given in the list below.

Among the next highest priorities was research aimed at the evaluation and improvement of the dose-response methods in this guidance document. In particular, the comparative potency method for whole mixtures and the interaction-based Hazard Index need to be demonstrated with different kinds of mixtures. Methods for validation of these two methods also need to be developed, followed by the validation exercise itself for several different mixtures.

The most often mentioned research area was uncertainty analysis. Each of the methods in this guidance document produces a single risk estimate. An initial goal is to present that risk estimate as a plausible range in addition to the single recommended value. A related goal is to present a range of risk estimates that reflects all the risk methods applied to the mixture of concern, i.e., the uncertainty in model selection. Data uncertainties should also be addressed, at least by sensitivity analysis. Subsequent efforts should pursue more complete uncertainty characterization, including methods for choosing the default distributions for the parameters and variables in each method. Uncertainty characterization is also one of the components of the Forum's cumulative risk framework project, so further work will commence in this area over the next few years.

The other main research needs raised during the authoring and review of this guidance document covered a wide range of scientific areas. The most commonly discussed topics are in the following list. The research areas are roughly grouped by scientific discipline or application.

<u>Exposure assessment</u>

- data and models for degradation over several years (e.g., pathogens in groundwater, pesticide mixtures in soil).
- models/data for chemical and biological interactions influencing mixture transport.
- mixture changes (chemical composition, relative proportions) from facility failures (e.g., drinking water, municipal combustors).
- procedures for artificial degradation or weathering of complex mixtures.
- procedures for monitoring mixtures when there are hot spots with each spot having a different driver chemical.
- biomarkers of exposure that are specific to single chemicals or chemical classes and mathematical models that relate the biomarker to existing or prior external exposure levels, and to tissue levels and/or tissue-specific toxic effects.

Statistical/mathematical methods

- formulas for incorporating independence when adding upper-bound risks ($n > 3$).
- concepts and methods for tolerance distributions for $n > 2$ chemicals.
- uncertainty analysis, i.e., Bayesian, Monte Carlo simulation for each of the mixture risk assessment procedures.
- efficient and stable numerical methods for modeling highly complex interacting systems (hundreds of chemicals, multiple tissues, time-variable exposures).
- statistical graphics methods for demonstrating and displaying interactions in multichemical mixtures ($n > 5$).

Biomathematical models

- models for describing the dependence of interaction magnitude on total dose and on component fractions.
- biologically based models that separate out the relative differences of chemicals in terms of pharmacokinetics and pharmacodynamics.
- models that incorporate aging and growth, and more physiological processes and factors than just flows to major organs and tissues.
- models for initiation-promotion interactions that include background exposures to initiators or promoters.

Human studies

- database of epidemiology studies with exposure-response information on mixtures.
- database of occupational health studies with exposure-response information on mixtures.
- methods for estimating interaction magnitudes in epidemiology studies that relate to (are consistent with) physiologic measures of interaction magnitude.
- information on background exposure levels, background prevalence of health conditions, and those population characteristics that indicate increased susceptibility to toxic chemicals, including models that quantify the influence of population characteristics on toxicology.

Toxicology

- modes and mechanisms of interaction for carcinogens.
- data describing the dependence of interaction magnitude on total mixture dose and on component fractions.
- concordance across animal species of specific toxic effects, modes of action, and modes of interaction.
- data and modes of interaction for inhibition (one chemical is nontoxic).
- data and concepts for particulate interactions with other airborne chemicals.

- more examples and methods for short-term whole-mixture toxicity testing, particularly data showing the representativeness of in vitro studies to represent in vivo toxicity.
- relationships between mode of toxic action and mode of interaction.
- concepts, mechanisms or modes of action, or toxicity data to explain the mathematical interaction models of proportional response addition and straight-line isoboles that are not parallel.
- interaction studies on major chemical classes to establish empirical interaction classes based on interaction patterns.
- test procedures that mimic real-world exposures (e.g., species-adjusted intermittent exposures to correspond to occupational exposure patterns)
- biomarkers of toxicity that are specific to single (or related) toxic effects and mathematical models that relate the biomarker to actual measurable toxic endpoints.

Risk methods

- development of screening assays for mixtures to identify combinations of chemicals that are most toxic or that potentially interact.
- risk estimation for a mixture of mixed types, including similar, independent, and interacting chemicals with same target organ, e.g., for classes with similar (RPF) chemicals and other chemicals.
- risk estimates or qualitative risk indicators for unidentified chemicals in a mixture (see U.S. EPA, 1998d. Comparative risk framework methodology and case study. SAB external review draft. NCEA-C-0135).
- MOE methods for carcinogens using response addition.
- RPFs from dose-response data on all chemicals, as improvement over HI because it allows actual estimate of toxicity from the index chemical's dose-response curve.
- use of interaction patterns for estimating interaction direction in a chemical class.
- methods for prioritizing chemical pairs (air, drinking water) for further study on the basis of health risk.
- methods for prioritizing complex mixtures for further study on the basis of health risk.
- methods for prioritizing complex mixtures for further study on the basis of degradation potential.

3. METHODS FOR WHOLE-MIXTURES DATA

3.1. INTRODUCTION

If whole-mixture data are available, then one approach to the health risk evaluation of a chemical mixture is to perform a risk assessment using health effect, dose response, and exposure data on the complex mixture. Health effect and dose-response data include human epidemiologic, clinical, or occupational studies; animal studies on the complex mixture; or in vitro data on the complex mixture. Exposure data include both environmental measurements and human activity patterns that take into account environmental fate, temporal patterns of exposure, and routes of exposure. The evaluation of whole mixtures in this document is subdivided into categories depending on data availability: data directly on the mixture of concern, data on a sufficiently similar mixture, or data on a group of similar mixtures.

3.1.1. 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 effect, dose-response, or exposure 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, 1989a, 1996a,d). Exposure and toxicity data on the mixture of concern are most likely to be available on highly complex mixtures such as coke oven emissions, which are generated in large quantities and associated with or suspected of causing adverse health effects. Issues that need to be considered in order to justify performing a risk assessment directly on the mixture of concern include bioavailability to humans of the mixture in the environment, stability or variability of the mixture composition over time, consistency of the mixture composition relative to its source, and potential differences between the mixture tested in the laboratory and the mixture found in the environment. These factors should be taken into account or the confidence in and applicability of the risk assessment is diminished.

3.1.2. Data Available on a Sufficiently Similar Mixture

If adequate data are not available on the mixture of concern, but health effects data are available on a similar mixture, a decision should 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" should be made on a case-by-case basis, considering not only the uncertainties associated with using data on a surrogate mixture, but also contrasting the inherent uncertainties if one were to use other approaches, such as component-based methods.

In determining whether a mixture is sufficiently similar, consideration should be given to any available information on the components that differ or are contained in markedly different proportions from the mixture of concern. In addition, if information exists on differences in environmental fate, uptake and pharmacokinetics, bioavailability, or toxicologic effects for either of these mixtures or their components, it should be considered in deciding on a risk assessment approach. If such information is not available, it should be identified as a source of uncertainty. If toxicity data for the sufficiently similar 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.

3.1.3. Data Available on a Group of Similar Mixtures

In some cases, data are available on a group of similar mixtures that are known to be generated by the same commercial process or emissions source, but that vary slightly in composition, depending on factors such as time since emission, environmental transformation, or geographic location of emission sources. Data are then 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 environmental fate data, chemical structures, and toxicologic data of the various mixtures (Section 3.4). If no significant differences are noted, then a risk estimate can be made by extrapolating across these similar mixtures by comparing toxicity across various assays (Section 3.3).

A group of mixtures may be considered similar if they have the same components but in slightly different ratios or have several common components but a little fewer or additional components. This judgment can be based on empirical measurements or on indirect evidence. The risk assessor should 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; and (5) establishing a common source of formation or emission for the group of mixtures.

3.1.4. Environmental Transformations for Whole Mixtures

A mixture's composition can change over time in the environment and thus become an issue for the development of a whole-mixture risk assessment. The impact of this phenomenon 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 (see Section 2.4 on exposure issues).

Whenever the mixture composition is affected by environmental factors, the uncertainty and possible bias in the resulting risk assessment should 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.

3.1.5. Uncertainties With Whole-Mixture Studies

Even if a risk assessment can be made using whole-mixture data, it may be desirable to also conduct a risk assessment based on toxicity data on the components in the mixture using procedures outlined in Chapter 4. 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 should 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 should be discussed. Additional evaluation may be warranted before developing the risk characterization.

3.2. WHOLE-MIXTURE RFD/C AND SLOPE FACTORS

3.2.1. Introduction

A dose-response assessment has been done by the Agency for several whole mixtures (see Sections 3.4.2 and 3.4.3 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 are used to derive the dose-response assessment can be considered either exactly the same or sufficiently similar. This concept of "sufficient similarity" 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 are 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, the dose-response assessment can be applied with high confidence. As the mixtures being compared become less similar, there would be less confidence in applying a dose-response assessment because the 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 exposure to the mixture of concern. Thus, the risk assessor should be able to apply dose-response assessments with confidence from highly similar mixtures, know the problems of applying them for less similar mixtures, and make some judgment about where on this continuum each case lies.

A dose-response assessment for a single chemical by an oral route of exposure may result in the calculation of a reference dose (RfD), defined on the Agency's Integrated Risk Information System (IRIS) as follows (U.S. EPA, 2000a):

> 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) (U.S. EPA, 1994a). 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 or a slope factor (U.S. EPA, 1987, 1996a). Data either on the mixture of concern or on a sufficiently similar mixture can be considered for developing these dose-response assessments with accompanying discussions of similarity judgment and uncertainty.

3.2.2. 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 1990s, with the resulting information made available on IRIS (U.S. EPA, 2000a). 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 database.

3.2.2.1. *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 threefold factor for limitations in the database, particularly relative to the issue of male reproductive effects; and a threefold 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 database 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 database 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."

3.2.3. 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, 2000a). 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 $\mu g/m^3$ air breathed, of 6.2E-4 per $\mu g/m^3$) was based on respiratory cancer in males exposed in an occupational setting to coke oven emissions. This assessment is different from most cancer quantitative assessments 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 direct exposure to either coke oven emissions by workers or to the emissions' 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.

3.2.4. 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. This procedure is essentially the same whether the available data are directly on the mixture of concern or on a sufficiently similar mixture. In the latter case, the risk assessor must support the similarity assumption in addition to following the single-chemical procedure. The difference for the mixture assessment lies in several areas: data requirements, the establishment of the stability of the mixture, cautions relative to dose-response models for mixtures data, discussions of the uncertainty relative to the mixture assessment, 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 their absence, a strong animal database, such as the primate data that were used for the Aroclors, 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) *Sufficient similarity (when available data are on a similar mixture):* 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, using the criteria discussed in Section 3.1.2. The risk assessor must consider the number of components that are the same across the mixtures, the differences in their proportions, common modes of action across the mixtures or their components, and common sources of formation or emission for the group of mixtures. Whatever judgment is made must be supported by the risk assessor.

(4) *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, 1996d, 1999). The approaches recommended in the Proposed 1996 Cancer Guidelines (U.S. EPA, 1996a) 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 procedure). 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 modes of action of the toxicity of single compounds, and may not be as well justified when applied to the mixture as a whole.

(5) *Guidance on the uncertainties and 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 are diminished. The confidence in the assessment must be discussed, along with any cautions relative to its use in risk characterizations (see example in Section 3.2.2 for Aroclor 1016).

3.3. COMPARATIVE POTENCY

3.3.1. 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 that 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 will 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 would 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.

3.3.2. 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., 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:

$$\{X_i\} = \text{group of } m \text{ similar mixtures, where } i = 1,\ldots,m \tag{3-1}$$

$$\{Aj\} = \text{the group of } n \text{ bioassays, where } j = 1,\ldots,n \tag{3-2}$$

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

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

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.

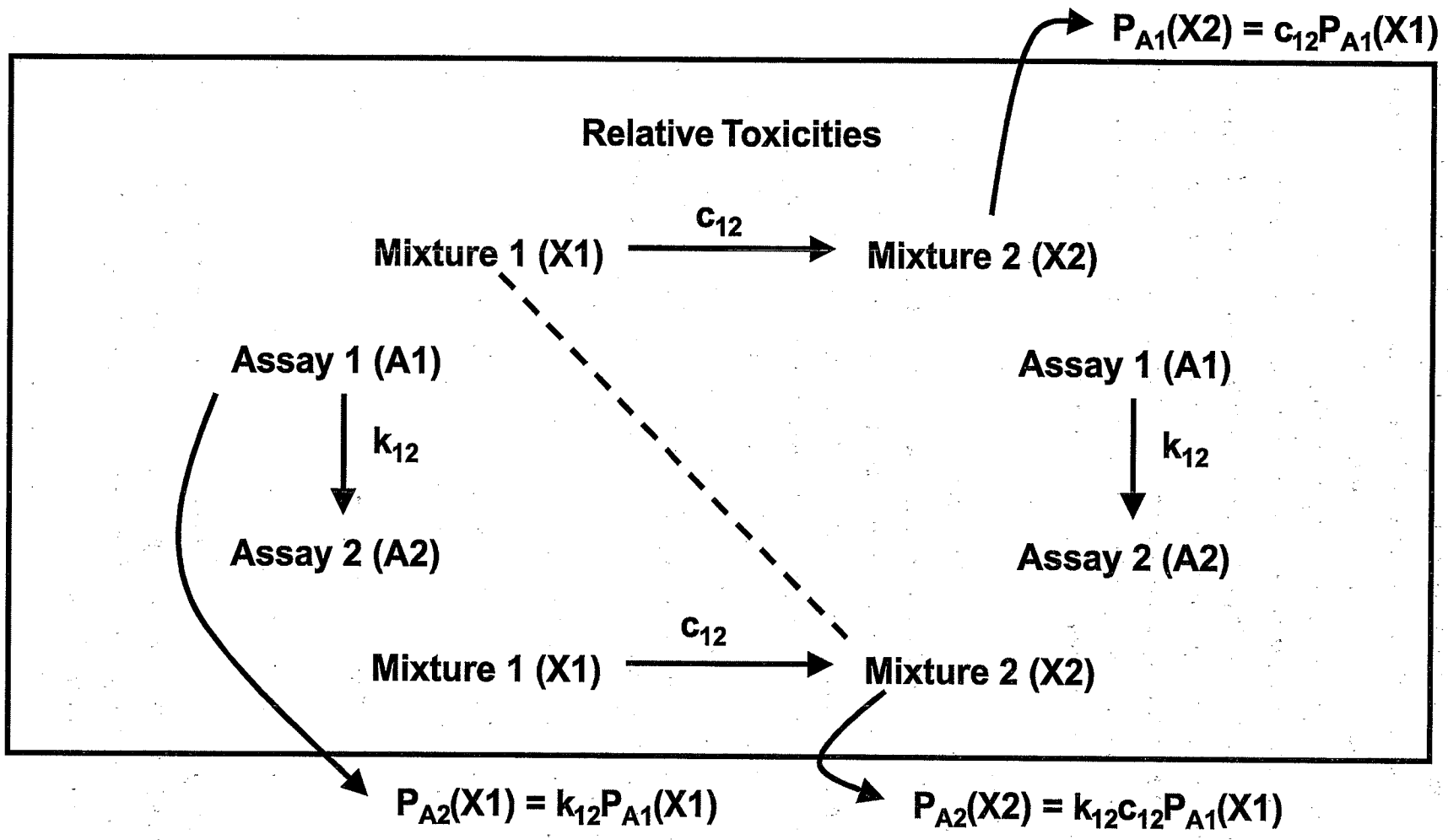


Figure 3-1. Proportionality assumption for two assays and two mixtures.

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}:

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

3.3.2.1. *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.

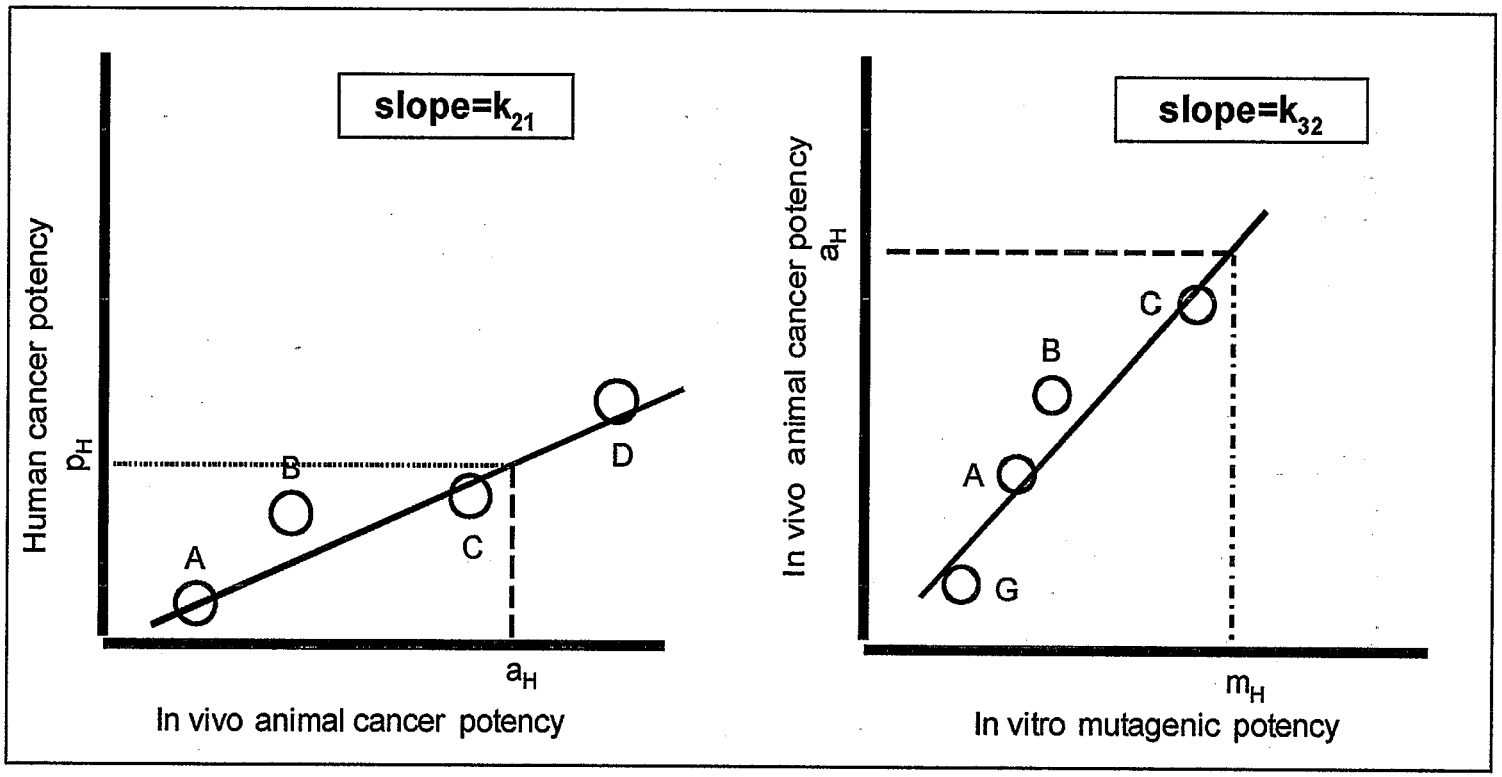
The human cancer potency for X_2 is then estimated as follows. First, k in Equation 3-3 (or k_{12} in Figure 3-1) can be estimated to be 2. Because X_2 is a member of the similarity class that includes mixtures X_1, X_3, and X_4 , the same cross-assay ratio holds for X_2 as for all the other similar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

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

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.

3.3.2.2. *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:



Hypothetical comparative potency example using proportionality constants with two assays.
(Left) Human potency estimated from animal data for four mixtures.
(Right) Animal potency estimated from in vitro data for four 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 - three assays.

- 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 Figure 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 Figure 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:

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

As shown in Figure 3-2, the two graphs can be used together as a nomogram where the potency of H on A1 is plotted from its potency on A3 (see dashed lines in the figure). Note that because data for H exist only 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.

3.3.2.3. *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). This example is only presented to illustrate the application of the comparative potency method. The unit risk estimates presented here are those published and do not necessarily represent the current EPA risk estimates for the chemicals involved.

The data for this example 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 ($k = 4 \times 10^{-4}$) 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

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	9.3×10^{-4}
Roofing tar	0.40	3.6×10^{-4}
CSC	0.0024	2.2×10^{-6}
Diesel	0.31	$(0.7 \times 10^{-4})^d$

[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.

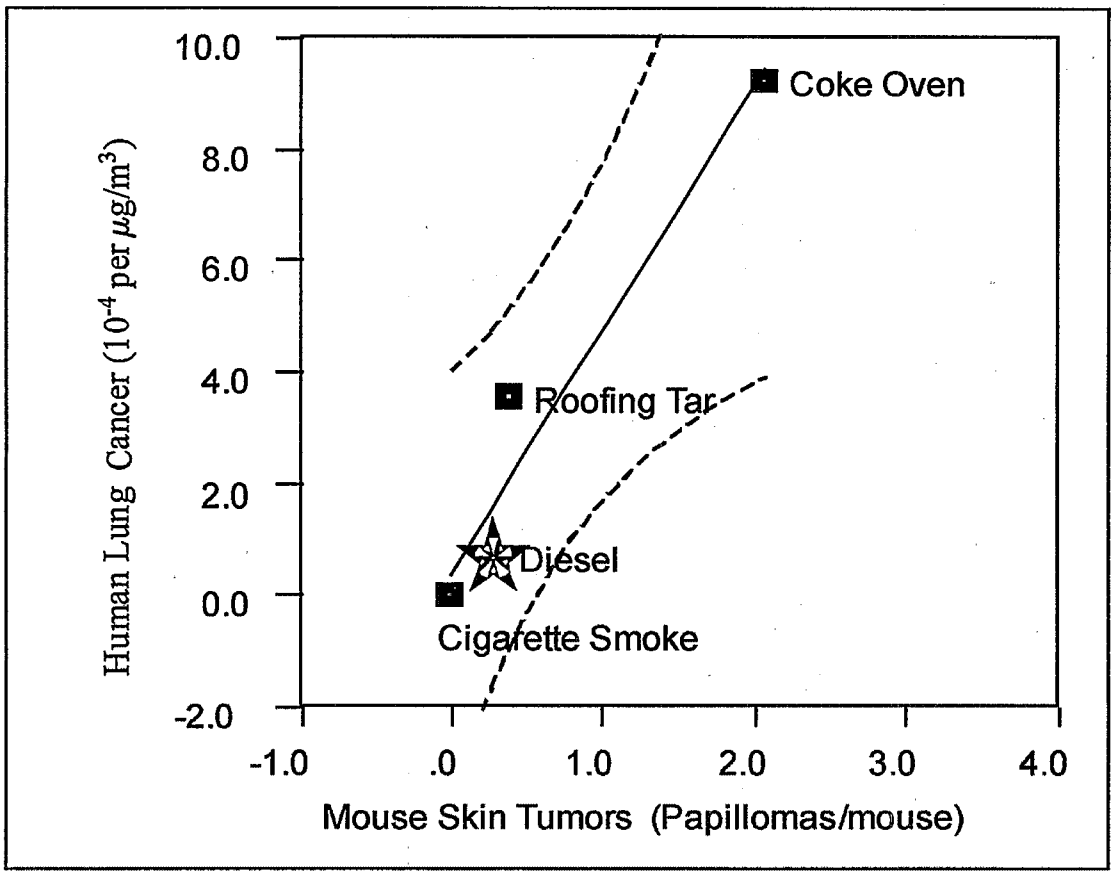
variability. For our purposes, however, with only three points, a more relaxed significance level (type I error rate) (e.g., $\alpha = 0.20$) may well be good enough. So we could substitute this value of k in Equation 3-3 to get:

$$P_{A1}(diesel) = (4 \times 10^{-4}) * P_{A2}(diesel). \qquad (3\text{-}7)$$

This estimate using comparative potency compares reasonably well with an estimate of 0.7×10^{-4} derived by traditional single-substance methods from rodent data (Table 3-1).

3.3.2.4. *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} versus 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.



Linear regression shown with 95% confidence bands based on nondiesel data.

Figure 3-3. Combustion mixtures (PAH).

Data from Nesnow (1990).

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.

3.3.3. 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:* Describe the degree of consistency within the mixture group of the cross-assay potency 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.

3.3.3.1. *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 or biologic 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; and (5) establishing a common source of formation or emission for the group of mixtures. Although there are references to the use of comparative potency for endpoints other than cancer (Albert,

1985), the methodology has been used by EPA only for cancer potency prediction. Use of comparative potency for noncancer 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 *modi 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.

Another potential screening method for similarity of mixtures is to examine the similarities of individual major chemical components by activity profile and/or structure-activity relationships (SAR) analysis. Nesnow (1990) suggests that EPA's genetic activity profile (GAP) software can be used to identify structurally and or biologically similar chemicals (Waters et al., 1988a,b). The OncoLogic Cancer Expert System developed for EPA (Woo et al., 1995a) can be

used to screen for structurally and/or functionally similar chemicals with respect to carcinogenicity as the toxicity endpoint. 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 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 et al., 1990). Note that these SAR models are limited in that they only generate predictions for single chemicals, which must be extrapolated to infer similarity among a group of mixtures.

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 the 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 subclass than across the whole universe of combustion mixtures or with another combustion subclass (e.g., tobacco smoke condensates).

3.3.3.2. *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 that 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, and development of preneoplastic 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 that 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 that 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) (Appendix A) and subsequent guidelines for carcinogenicity, developmental toxicity, reproductive toxicity, and neurotoxicity (U.S. EPA, 1996a, 1991a, 1996b, and 1998b, 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 3.3.2.3). 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 that 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, 1996a). 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 those 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, POMs, 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).

3.3.3.3. *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 3.3.2.3.

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

3.4. ENVIRONMENTAL TRANSFORMATIONS

3.4.1. Using Environmental Process Information to Determine Mixture Similarity

Environmental processes can affect the exposure, and thus the toxicity, of a mixture in the environment, so one approach to a whole-mixture assessment is to adjust the risk assessment based on what is known about the mixture because of environmental transformations. 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.

3.4.2. Procedures for Incorporating Environmental Process Information

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 on approaches for using environmental process information to determine mixture similarity, given certain data scenarios, are given below:

Data scenario/approach: *Toxicity information is available on most mixture component chemicals/use 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 4.4).

Data scenario/approach: *Toxicity information is available on only a few mixture components/use bounding estimates and 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 Hazard Index (Section 4.2), relative potency factors (Section 4.4), and whole-mixture testing (Section 3.1.5), 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.

3.4.3. Geographic Site-Specific Modifications: An Example Using PCB Mixtures

EPA's approach to assessing the cancer risk from environmental PCBs (U.S. EPA, 1996c; 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, whereas the relative potency approach is based on a large body of experimental information that elucidates modes of action or mechanisms of toxicity and quantifies their potency for a small number of PCB congeners that act like dioxin.

3.4.3.1. *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 3-2 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 were "12," and the last two digits indicated the percent chlorine by weight. Aroclor 1016, with approximately 41% chlorine, is an exception to this scheme.

3.4.3.2. *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 significantly increased incidences of liver tumors were found in female rats for all Aroclors and in male rats for Aroclor 1260 (Table 3-3). 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 (Table 3-4).

3.4.3.3. *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

Table 3-2. 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

- = less than 1%.
ND = not detected.
NR = not reported.
Sources: Compiled by U.S. EPA (1996c) from other sources.

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 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

Table 3-3. Liver tumor[a] incidences for Aroclor mixtures			
Mixture	**Dose**	**Females**	**Males**
Aroclor 1260	Control[b]	1/85 (1%)[c]	7/98 (7%)[c]
	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%)[c]	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%)[c]	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%)[c]	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%)

[a] Hepatocellular adenomas, carcinomas, cholangiomas, or cholangiocarcinomas in rats alive when the first tumor was observed.
[b] One control group supported all experiments.
[c] Statistically significant ($p<0.05$) by Cochran-Armitage trend test.

Source: Brunner et al., 1996, reported by U.S. EPA, 1996c.

Table 3-4. Human slope estimates (per mg/kg-day) for Aroclor mixtures		
Mixture study	**Central slope**	**Upper-bound slope**
1016, Brunner et al., 1996	0.04	0.07
1242, Brunner et al., 1996	0.3	0.4
1254, Brunner et al., 1996	1.2	1.5
1260, Brunner et al., 1996	0.4	0.5
1260, Norback, 1985	1.6	2.2

Source: U.S. EPA, 1996c.

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 3-4 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 3-4 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.

3.4.3.4. *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 non-dioxin-like-like modes of action, it is important to consider the contribution from both dioxin-like and non-dioxin-like-like modes of action to the total risk. Risks for the dioxin-like and non-dioxin-like-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 modes of action. For the non-dioxin-like-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 (1996c) provides a detailed example of these calculations.

3.4.3.5. *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 the 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.

4. METHODS FOR COMPONENT DATA

4.1. INTRODUCTION

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 response-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. Dose-additive models may be an adequate default procedure for chemicals affecting the same target organ, but 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 dose-response model should be used.

The mixtures methods in this chapter rely heavily on existing EPA risk assessment information on single chemical toxicity, such as that in the EPA IRIS files. Levels of exposure for the mixture component chemicals are assumed to be estimates obtained following the appropriate Agency exposure assessment guidance (e.g., U.S. EPA, 1992). The procedures and terminology associated with dose response and risk characterization for single chemicals, such as the RfD, RfC, and cancer potency values, have the same interpretation in the mixture procedures in this chapter. 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.

4.1.1. Criteria for Dose Addition vs. Response Addition

Toxicologic interactions are defined in this guidance document (Appendix B) to facilitate the selection and application of specific risk assessment methods. When adequate evidence for toxicologic interactions is not available, the most appropriate no-interaction approach (dose addition or response addition, as detailed below) will be employed. Toxicologic "interactions" are then operationally defined by mixture data showing statistically or toxicologically significant deviations from the "no-interaction" prediction for the mixture.

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 (1991) 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 modes of action; 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 straightforward and in common use, and because hypothesis tests exist to determine whether data are consistent with each of these concepts (see Gennings, 1995; Gennings and Carter, 1995). These definitions do not indicate specific toxicologic modes of action, although they should be consistent with the major examples and concepts of toxicologic interaction. Dose addition and response addition then represent default approaches for toxicologically similar and toxicologically independent chemicals, respectively. 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 combinations of dose and response addition as well as toxicologic interactions. Information on interactions can be included as modifications of the "no-interaction" approach that was selected (see Sections 4.3 and 4.5.4).

The primary criterion for choosing from dose or response addition as the no-interaction approach is the similarity or independence among the chemicals in the mixture. This judgmental decision, detailed further in Sections 4.1.1.1 and 4.1.1.2, 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. If tissue levels can be estimated, then the judgment of similarity or independence can focus on the toxicologic mode of action. If external exposure levels are used instead of tissue doses, then the judgment of toxicologic similarity or independence must consider all the processes from contact with the environmental media to the toxicity itself (i.e., uptake, metabolism, distribution, elimination, and toxicologic mode of action). 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).

4.1.1.1. *Dose Addition*

In the simplest terms, two chemicals are dose additive if chemical B is functionally a clone of chemical A. In this ideal case, 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 characterization of dose addition requires a constant proportionality between the effective doses of the two chemicals. This means that, for equal effects, the dose of chemical B is a constant multiple of the dose of chemical A. The dose-response functions are then congruent in shape. Let t be the proportionality constant that denotes the relative effectiveness of chemical B to chemical A as estimated by the ratio of their iso-effective doses, e.g., their ED_{10}s. Let p_1 and p_2 be response measures and f(d) and g(d) be the dose-response functions for chemicals A and B, respectively. Then for doses d_1 and d_2 of chemicals A and B, respectively, we have:

$$p_1 = f(d_1), \tag{4-1}$$

$$p_2 = g(d_2) = f(t * d_2) \tag{4-2}$$

The last equation (4-2) illustrates dose addition by converting dose d_2 into an equivalent dose of chemical A and then using the dose-response function f of chemical A to predict the response. For a mixture of the two chemicals, the mixture response p_{MIX} is then given in terms of the equivalent dose and dose-response function for chemical A:

$$p_{MIX} = f(d_1 + t * d_2) \tag{4-3}$$

Among the many ways to decide dose-addition, the *isobole* is one of the more common graphical methods (see Figure 4-1). 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 A and the y-coordinate is the dose of chemical B 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 4-1, 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 A and 50 mg/kg of chemical B will yield a 10% response in the test animals. Note that this decision tool can be applied to any fixed response measure, whether percent responding in a

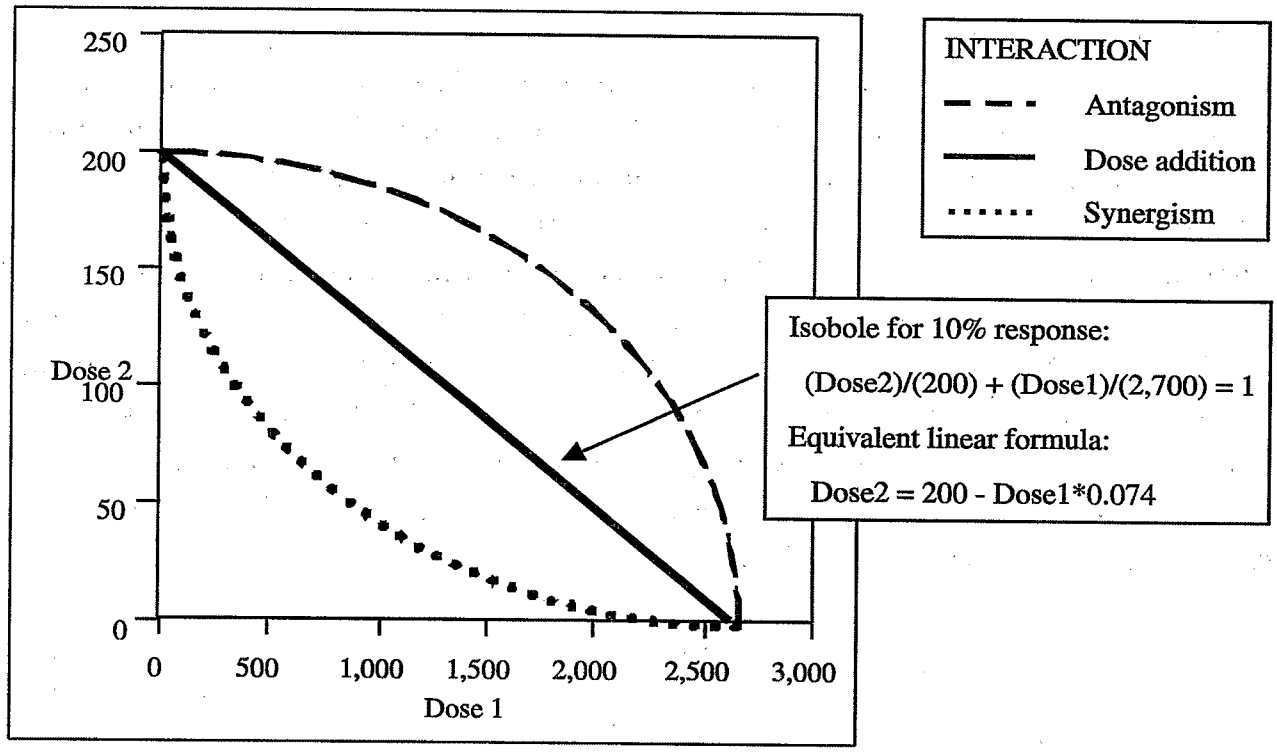


Figure 4-1. Isoboles for 10% response level of combination doses (d1, d2) of two chemicals showing the possible types of interaction.

group, deficit of functionality, severity of a lesion, or any measure of toxicity that is constant along the isobole.

When the set of equal-response points is a straight line, the two chemicals are said to be dose-additive. Although in Figure 4-1 the other two isoboles show clear curvature, in many plots the nonlinearity is less obvious. Statistical methods exist that help in deciding whether the points indicate a departure from dose additivity (Gennings, 1995), and their use is strongly recommended. Note that in the simple "clone" definition of dose addition, all isoboles for different response rates will be parallel. Other more general definitions of dose addition have also been proposed (Svendsgaard and Greco, 1995), including 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 only reflect doses associated with unacceptable toxicity (e.g., LD_{10}s) or exposure levels much higher than the environmental

levels of concern, then justification must be given for extrapolating the dose-addition property to the 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 modes of action, 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 modes of action showed consistency with dose addition when each chemical was at or slightly below its NOAEL. Similarity of toxic modes of action 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 RPF method, the TEF method, which is a special case of the RPF method, and the HI method. They differ in the required knowledge about toxicologic processes 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 apply 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. This equivalent exposure is the exposure level of the index chemical that elicits the same response as the mixture exposure. The risk assessment then evaluates the equivalent index chemical exposure on that chemical's dose-response curve in order to predict the mixture response.

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 Hazard Index method requires only similarity in target organ. As with the general RPF method, a separate Hazard Index 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 Hazard Index is interpreted by whether or not it is greater than 1. The scaling factors for the Hazard Index are based only on each component's toxicity, preferably related to the target organ being assessed so that the interpretation of the Hazard Index value can be tied to the target organ risk. For example, if the ED_{10} for liver effects is used (so that $1/ED_{10}$ is used as the toxicity scaling factor), then when HI=1, the mixture is at its ED_{10} 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 Hazard Index 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 Hazard Index method, the Hazard Index 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.

4.1.1.2. *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 4-1 through 4-3, the statistical law of independence is, for two chemicals:

$$p_{MIX} = 1 - (1 - p_1) * (1 - p_2) \tag{4-4}$$

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

$$p_{MIX} = p_1 + p_2 - p_1 * p_2 \tag{4-5}$$

which, for small single-chemical responses and only two chemicals in the mixture, is well approximated by the simple summation:

$$p_{MIX} = p_1 + p_2 \tag{4-6}$$

The generalization of Equation 4-4 to an arbitrary number (n) of chemicals is:

$$p_{MIX} = 1 - (1 - p_1)*(1 - p_2)*\ldots*(1 - p_n) \tag{4-7}$$

Unless the number of mixture components is small and the individual risks are very small, Equation 4-7 should be used for the response addition mixture estimate.

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. For example, if 20 mg/kg of chemical A causes a 5% increase in liver weight and 30 mg/kg of chemical B causes a 3% increase, then the prediction for a mixture of 20 mg/kg of A and 30 mg/kg of B is a liver weight increase of 8%. 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 for general use 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 B). 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.

4.1.1.3. *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, consider the case where decreasing the exposure reduces the probability of an effect, but not its

severity (as EPA traditionally assumed for carcinogens). Simultaneous exposure to several of these chemicals could then accumulate many small risks and be unacceptable in combination even though the individual risks were acceptably small.

In contrast, consider the case where decreasing exposure results in a decrease in toxic severity so that there is a practical threshold below which the effects are considered nonadverse. If these chemicals are toxicologically independent and at individual exposure levels below their respective practical thresholds, then an assessment of simultaneous exposure to several of these chemicals may conclude there is 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.

In some cases, the sensitivity or resolution of the toxicity test may be worse as exposure level decreases. In such cases, the exposure level labeled as an apparent toxicity threshold may only reflect the reduced ability to discern that dose-related toxicity has occurred. Any risk assessment based on evaluations near these practical thresholds should reflect the uncertainty caused by the reduced sensitivity or resolution of the underlying toxicity tests. When quantitative corrections are not possible, the risk characterization must include these study weaknesses in the discussion of uncertainties.

4.1.1.4. *Evidence for Dose or Response Additivity*

Several studies have been published that suggest that dose or response additivity adequately characterizes mixture risk. The large variety of possible mixtures, however, precludes any strong characterizations of the accuracy of additivity methods. Some sense of the opinion of toxicologists, however, can be gained from some key publications, in which dose or response addition is recommended as a plausible default procedure. Ikeda (1998) 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. The NAS book (NRC, 1988, p. 100) on complex mixtures is less precise. NAS 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 mixture risk assessment (U.S. EPA, 1986) (Appendix A) 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.

Reviews of toxicologic interaction studies do not often evaluate additivity, or are not able to develop general conclusions. In too many cases, a study was not designed properly for detecting departures from additivity. For example, in a review of statistical methods in 462 interaction studies (U.S. EPA, 1990), roughly one-third of the reported results indicated no interaction or some kind of additivity, but nearly half of the studies used no statistical analysis or did not report what procedures were used in determining statistical significance. As a result, it is presently difficult to guess how common some kind of additivity might be for pairwise interactions.

The decision to use dose addition and response addition as default "no-interaction" definitions is primarily based on scientific plausibility when their assumptions are met (i.e., toxic similarity for dose addition, independence for response addition). In addition, these default approaches have clarity, simplicity, and ease of implementation. The evidence for either dose addition or response addition as a good approximation for a mixture risk assessment is not strong, and clearly is not comprehensive in representing the varying types of chemicals considered in environmental risk assessment. 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.

4.1.2. Toxicologic Interactions

Regulatory decisions usually involve the assessment of chemical mixtures, though often on a chemical-by-chemical basis. Typical exposures, in contrast, are composed of a combination of biological, chemical, and physical agents that may influence each other's adverse effects. 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 (1991) 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 B-2 (Appendix B) recommends a set of definitions for use in chemical mixture risk assessment. It clarifies the terminology related to additivity and interaction effects for both cancer and noncancer endpoints.

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 C). The impact of the joint exposure 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). It must be emphasized that antagonism is not the same as inhibition. Antagonism only implies a lesser joint response than predicted from dose addition. Presence of antagonism does not justify lowering of risk estimates of an affected chemical, say by increasing its Reference Dose. An antagonistic chemical is also toxic. In contrast, the inhibitor chemical is not toxic by itself, but does reduce the toxicity of the second chemical. Only for inhibition could risk levels for the second chemical be adjusted because of reduced toxicity. Additional information and examples of data on interactions can be found in Appendix C.

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, sex, 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 is imperative. An evolving approach is the utilization of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling, coupled with model-oriented toxicology experiments (Tardif 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 PBPK/PD modeling and the metabolic processes have been presented in Appendix C.

Evidence of toxicologic interaction should be reflected in the mixture risk assessment (U.S. EPA, 1986). Previous risk assessments of multichemical exposures by 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 cleanup goals and the estimated risk, however, would not change. Consequently, most mixture 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 chapter is new. Its use is encouraged so that EPA can gain experience regarding the difficulties and advantages of an interaction-based approach and then identify ways to improve the approach.

In developing an interaction-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.

4.1.3. 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 4.5).

4.1.4. 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.

4.1.4.1. *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 suggests 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.

4.1.4.2. *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. In the review cited previously on available interaction studies (U.S. EPA, 1990), statistical tests were used to decide the presence of toxicologic interaction, but dose-response models for interactions were extremely rare. For example, of the 462 studies reviewed, only four gave a prediction under no interaction (using response addition as the default). 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 et al., 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 only weakly supported by empirical studies.. Based on a review of EPA's Mixtox database (U.S. EPA, 1990), reflecting 437 articles on interactions between pairs of environmental chemicals, many studies 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 questionable. 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 methods described in Section 4.3 (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 EPA and ATSDR.

Toxicologic understanding of interaction is also limited. Although interaction modes of action 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.

4.1.4.3. *Presenting Component-Based Risk Characterization*

The consequence of this early stage of mixture 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 this chapter are developed from available concepts and data on simple mixtures, all component-based quantitative mixture 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. For example, 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 as subjective choices in modeling (e.g., significance levels for inclusion of model terms, confidence levels for creating interval estimates, levels for deciding adequate goodness-of-fit), 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.

4.2. HAZARD INDEX

4.2.1. Definition

The primary method for component-based risk assessment of toxicologically similar chemicals is the Hazard Index (Teuschler and Hertzberg, 1995), which is derived from dose addition (Svendsgaard and Hertzberg, 1994; also see Sections 2.6.1 and 4.1.1). In this guidance document, dose addition is interpreted as simple similar action (Finney, 1971), where the component chemicals act as if they were dilutions or concentrations of each other differing only in relative toxicity. Dose additivity may not hold for all toxic effects. Further, the relative toxic potency between chemicals may differ for different types of toxicity or toxicity by different routes. To reflect these differences, the Hazard Index is then usually developed for each exposure route of interest, and 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 route and one toxic effect or target organ.

The Hazard Index is defined as a weighted sum of the exposure measures for the mixture component chemicals. The "weight" factor according to dose addition should be a measure of the relative toxic strength, sometimes called "potency." Because the Hazard Index is tied to dose addition, each weight factor should be based on an isotoxic dose. For example, if the preferred isotoxic dose is the ED_{10}, then the Hazard Index would equal the sum of each chemical's exposure level divided by its ED_{10} estimate. 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, a Hazard Index for liver toxicity should 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 similarly shaped 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 4.1.1 for other definitions and for more background information). When the response involves quantal data on the number of animals (people) responding, the evidence for dose addition can also include parallel log dose-probit response curves of the component chemicals. 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, mode of action, 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 Hazard Index method is specifically recommended only for groups of toxicologically similar chemicals that all have dose-response data. 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). Additional information on mode of action or on other factors that could affect tissue exposure (e.g., deposition pattern in the nose) should be reviewed to ensure that dose additivity is 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 2.6.2 and 4.5). 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 Hazard Index 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. Clearly, all scaling factors in the same Hazard Index should reflect the same toxicity measure (e.g., $1/ED_{10}$). There is no commonly accepted standard measure of toxicity for exposure levels associated with minimal toxicity, in contrast to the slope factor for cancer (when

nonthreshold, low-dose linearity is assumed) or the LD_{50} for lethal levels. To ensure consistency with other EPA guidance on risk assessment, lethal dose data are not recommended for use in mixture risk assessment. The approach taken in the 1986 mixture guidelines (U.S. EPA, 1986) (Appendix A) for the scaling factors in the Hazard Index 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 Hazard Index is then quite general:

$$HI = \sum_{i=1}^{n} \frac{E_i}{AL_i} \tag{4-8}$$

where

E = exposure level,
AL = acceptable level (both E and AL are in the same units), and
n = the number of chemicals in the mixture.

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

$$HI = \sum_{i=1}^{n} \frac{E_i}{RfD_i} \tag{4-9}$$

where

E_i = daily oral intake of the ith chemical, and
RfD_i = EPA Reference Dose for the ith chemical.

Each term in Equation 4-9 is called a hazard quotient (U.S. EPA, 1989a) 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, 1994a).

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 a Hazard Index 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 Hazard Index is calculated for some different, less sensitive effect, the RfD will be too low, so the factor (1/RfD) will overestimate the relative toxicity and the Hazard Index will be too large. One alternative that avoids this critical effect conservatism is to use a toxicity-based exposure level 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 Hazard Index would be identical to Equation 4-9, with the TTD replacing 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 currently there is no official EPA activity deriving these values, as there is for the RfD and RfC. This alternative should be considered when there is sufficient reason to believe that the overestimate of the Hazard Index 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 database 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 HI. This discussion and recommendations also apply to HIs for shorter exposures, and to TTCs as replacements for RfCs in an Hazard Index 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 was the effect of concern, so the TTD and RfD were the same. This example illustrates that, for some endpoints, the substitution of the TTD will produce a Hazard Index value that is significantly less than the Hazard Index based on RfDs alone, while for others the difference is minor. In this example, the Hazard Index for reproductive effects changes from 3 to 1 by substituting the TTDs for the RfDs, whereas the Hazard Index 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 scaling factor. 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, 1996d). 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 involves 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 Hazard Index 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 Hazard Index 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. If the BMD is defined as the ED_{10}, then when HI=1, the mixture exposure should produce a 10% response (see Section 4.2.6, Equation 4-12).

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.

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.

4.2.4. Comparison of the Hazard Index Formulas

The four approaches to calculating the Hazard Index can be compared by whether they have various desirable characteristics. None of the approaches possesses all the desirable traits, so the preferred method will need to be judged for every application.

One of the key desirable features is the constraint to use only data on the effect of concern. Because the Hazard Index is tied to a specific effect, the underlying data should be on that effect. Substituting data on the critical effect introduces an unknown degree of conservatism, so that the Hazard Index is inflated by an unknown amount.

Another desirable characteristic is the use of statistical analysis on the entire dose-response study data, e.g., to generate a BMD. Statistical analysis of the dose-response data allows quantification of uncertainty and reflects more information by using the entire dose-response data set. Restriction to an actual experimental dose, such as focusing on a single NOAEL or LOAEL, ties the precision of the HI to the dose spacing used in the study. Also, when only the actual exposure level is used, there is no reflection of its statistical uncertainty in the HI calculation.

A third desirable characteristic is the constraint to use only data on humans for the exposure scenario of concern. As more extrapolation is performed, such as using an uncertainty factor to allow subchronic data to be used for a chronic risk assessment, the interpretation of the HI becomes more vague. Uncertainty factors play an important role in standardizing the data so that chemicals with different kinds of dose-response data can still be combined in the HI calculation. Because uncertainty factors are judgmental, not statistically derived scaling factors, their accuracy and precision are difficult to quantify.

Finally, it is important to have ready access to the data required for the particular approach. Whereas direct human dose-response data are preferred, they are rarely available for environmental chemicals. Similarly, although the TTD avoids the conservatism of the critical effect, and may use fewer uncertainty factors than the RfD, there are no plans within EPA for development of TTDs.

The four approaches can be summarized in Table 4-2. For easier comparison, only oral-exposure nomenclature is used. For inhalation, each "D" (for oral dose) in the column headers should be replaced by a "C" (for air concentration). BMD-hu refers to a BMD-based HI using human data for the exposure scenario of concern. TTD-BMD refers to the TTD-based HI where the TTDs use dissimilar BMDs that have been standardized by uncertainty factors.

The default procedure for the HI has traditionally been to use the RfD or RfC (U.S. EPA, 1989a). Because of their much wider availability than TTDs, standardized development process including peer review, and official stature, the RfD and RfC are recommended for use in the default procedure for the HI. When possible, the other methods should be employed, even if only

Table 4-2. Comparison of HI methods				
Feature	**BMD-hu**	**TTD-BMD**	**TTD**	**RfD**
Toxic effect of concern	yes	yes	yes	not usually
Statistical analysis of full dose-response data set	yes	yes	no	no
Species and exposure scenario of concern	yes	no	no	no
Easily available data	no	not much	some	yes

for some of the mixture components, to allow at least partial characterization of the uncertainty and conservatism introduced by use of the RfD or RfC.

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 HI should be calculated for each toxic effect of concern (U.S. EPA, 1986, 1989a). 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 HI 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 interaction-based HI (see Section 4.4).

When any effect-specific HI exceeds 1, concern exists over potential toxicity. Some research suggests that concordance across species of the sequence of target organs affected with increasing dose (e.g., the critical effect) and concordance of the modes of action are variable and should not be automatically assumed (Heywood, 1981, 1983). Some effects, such as hepatic toxicity, are more consistent across species, but more research is needed in this regard. 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 so specific effects should not be inferred unless considerable empirical or 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.

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.

No specific decision threshold is proposed for general application of 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.

4.2.6. Reference Value for a Mixture

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. A fish RfD can then be calculated as the level that represents the intake of fish (e.g., kg of fish flesh per day) associated with minimal risk.

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 0.05 as the fixed response for scaling the component doses:

$$1 = d_1/D_1 + d_2/D_2 \qquad (4\text{-}10)$$

where:

d_i = dose of ith chemical, and

D_i = dose of ith chemical that produce the response of 0.05.

In Berenbaum's equation, each dose is scaled according to "doses isoeffective with the combination." In this example, the "effect" is defined as a small response value, say 0.05. Then the D_i values are the respective ED_{05} values for the two components when exposure is to one chemical at a time. If the component doses are such that Equation 4-10 is true, then the mixture dose, $d_m = (d_1 + d_2)$, is at its ED_{05}, denoted here by D_m. This is determined by representing the joint exposure by fractions (f_i) of total mixture dose (i.e., $d_i = f_i * D_m$):

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

Dividing by D_m gives:

$$1/D_m = f_1/D_1 + f_2/D_2 \tag{4-12}$$

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

A similar procedure can be used to determine the reference dose for the mixture (RfD_m) by interpreting the isoeffective doses to be RfDs (i.e., doses producing negligible risk of adverse effects). If we invert Equation 4-12 and substitute the component RfDs for the component ED_{05}s, then we obtain:

$$RfD_m = 1 / (f_1/RfD_1 + f_2/RfD_2) \tag{4-13}$$

Example. Let the single chemical data be:

	Chemical 1	Chemical 2
RfD	20	35
Fraction in mixture	0.7	0.3

Then application of Equation 4-13 gives the mixture RfD as:

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

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 4.1.4).

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 database. As noted in the discussion of the HI (Section 4.2), RfDs (and RfCs) for different chemicals are derived separately, and often represent differing degrees of quality and relevance. Interpreting the overall quality of the mixture RfD as the composite of several variable-quality individual RfDs 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.

4.3. INTERACTION-BASED HI

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 (Hertzberg et al., 1999). The method follows an obvious approach: to begin with the dose-additive HI, and then modify its calculation to reflect the interaction results, using plausible assumptions to fill in the data gaps. A secondary assumption is that the influence of all the toxicologic interactions in the mixture can be adequately approximated by some function of the pairwise interactions.

4.3.1. HI Definition

4.3.1.1. *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.

EPA previously developed a weight-of-evidence procedure that uses binary interaction data to modify the HI (Mumtaz and Durkin, 1992; Mumtaz et al., 1998). This procedure reflected the strength of the available interaction studies as well as the amounts of each component in the mixture. The first step entailed 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 was then assigned a numerical binary weight-of-evidence (BINWOE) score. The BINWOE was 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:

$$HI_I = HI_{ADD} * UF_I^{WOE_N} \quad (4\text{-}14)$$

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) has been 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 the approach is 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 lack support from empirical assessments of the influence that some key experimental variables have on the interaction consistency. Further, the formula itself (Equation 4-14) 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.

The recommended procedure incorporates several changes from the original developed by Mumtaz and Durkin (1992). The main difference is seen in the formula (Hertzberg et al., 1999). Instead of the additive HI (Equation 4-9 in Section 4.2) being modified 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 a HI for liver toxicity. The Hazard Quotient (HQ_i) for the ith 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, indicated by the value of HQ_i, 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 (Krishnan et al., 1994).

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 interaction-based 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.

4.3.1.2. *Formula*

The interaction-based HI 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-based 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.

The WOE procedure modifies each HQ in the formula for HI. For the ith 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 i not equal to j). This multiplier is (each term is described below):

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

The full modified formula for the interaction-based HI, HI_{INT} , is then:

$$HI_{INT} = \sum_{i=1}^{n} (HQ_i * \sum_{j \neq i}^{n} f_{ij} \; M_{ij}^{B_{ij}\theta_{ij}}) \qquad (4\text{-}15)$$

where:

HI_{INT} = HI modified by binary interactions data,

HQ_i = hazard quotient for chemical i (unitless, e.g., daily intake/RfD),

f_{ij} = toxic hazard of the jth chemical relative to the total hazard from all chemicals potentially interacting with chemical i (thus j cannot equal i),

M_{ij} = interaction magnitude, the influence of chemical j on the toxicity of chemical i,

B_{ij} = score for the strength of evidence that chemical j will influence the toxicity of chemical i, and

θ_{ij} = degree to which chemicals i and j are present in equitoxic amounts.

Many formulas could be derived that reflect these ideas. The above formula 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 HI 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 Equation 4-15, the interaction-based HI for the mixture.

4.3.1.3. *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 current weight-of-evidence classification is given in Table 4-3. 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. Similar 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 4-3, 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. Although 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

Table 4-3. Modified weight-of-evidence classification[a]	
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 is: A. Insufficient to determine the direction of any potential interaction. B. Insufficient to determine whether any interaction would occur. C. Adequate as evidence that no toxicologic interaction between/among the compounds is plausible.

[a]See text for more detailed descriptions of each category.

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 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 method and the BINWOE method originally described in Mumtaz and Durkin (1992).

The term B_{ij} is simply the quantitative weight assigned to the qualitative WOEs (Table 4-4). 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.

4.3.1.4. *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 f_{ij}:

$$f_{ij} = \frac{HQ_j}{(HI_{add} - HQ_i)} \tag{4-16}$$

where HI_{add} is the standard HI 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.

Table 4-4. 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

4.3.1.5. *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}, co-workers (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 in either direction (0.2 = 1/5). 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.

4.3.1.6. *Weighting Factor for Relative Proportions (θ)*

The term θ_{ij} 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 ith and j^{th} components are said to be equitoxic if $HQ_i = HQ_j$. A measure of the deviation from equitoxic amounts for the ith and j^{th} components is defined simply as the ratio θ_{ij} of the geometric mean to the arithmetic mean:

$$\theta_{ij} = \frac{(HQ_i \bullet HQ_j)^{0.5}}{[(HQ_i + HQ_j) \bullet 0.5]} \qquad (4\text{-}17)$$

Note that as HQ_i approaches HQ_j, θ_{ij} approaches unity. As the difference between HQ_i and HQ_j increases, θ_{ij} approaches zero.

The term θ_{ij} 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).

4.3.1.7. *Example*

The properties of the interaction-based HI 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

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

factor of 5 for the route and duration of concern, with an interaction directly relevant to human health.

In scenario 2, each B_{ij} is equal to zero because the three chemicals are known to be additive (category IV-C in Table 4-3). 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 4-16 we see that regardless of the ratios of the components in the mixture, the sum of the f_{ij}s will equal 1.

In other words, the HI will not change from one based on additivity. The HI modified for interactions for scenario 2 is then:

Scenarios 1 and 3 are not quite as simple. Because these scenarios are identical except for the direction of the interaction (and hence the 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 4-15 yields an adjusted HI five times greater than the HI based on additivity. Note that in this simple case, both $B_{ij} = 1$ and $\theta_{ij} = 1$. Assuming that M is set to 5 (the proposed scenario says each chemical is known to potentiate the other by a factor of 5), then Equation 4-15 reduces to:

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

Thus, if the HI based on additivity were 1, the HI considering interactions would be 5. The counterpart, scenario 3, would give an interaction-based HI of 0.2.

Suppose, however, that the mixture of chemicals 1, 2, and 3 was such that the hazard quotients of each chemical were 0.98, 0.01, and 0.01, respectively. For such a mixture, it wouldnot seem reasonable to assume as great an interaction as in the equitoxic mixture because

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

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, $\theta_{ij} < 1$ for pairs involving chemical 1, resulting in a decrease in the interaction-based HI. For the effect of chemical 2 on chemical 1, using Equation 4-17 gives:

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

$$\theta_{12} = (0.98*0.01)^{.5} / (0.99/2) = 0.2, \quad f_{12} = 0.01 / (1.00-0.98) = 0.5$$

Thus, the partial adjusted hazard quotient for just the effect of chemical 2 on chemical 1 is:
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 4-15, 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 interaction-based HI 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 HI of 1, essentially the same as that under the assumption of additivity. Any time one chemical dominates the mixture composition to this extent, a good approximation is that the interaction-based HI 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 HI = 1 would have an interaction-based HI 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 interaction-based HI 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 4-4). The influence that this protective bias has on the interaction-based HI 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
Interaction-Based HI	3.3	0.45
n-fold increase or decrease of HI	3.3	2.2

4.3.2. Information Requirements

Empirical evidence of toxicologic interaction is required only for interactions of pairs of chemicals. Recall that one assumption of this procedure is that the mixture response can be adequately approximated by the modification of each term in the additivity-based HI using only pairwise interactions. The interaction-based HI, HI_{INT}, applies to one type of toxicity, so the interaction must influence that toxicity. For example, consider the case where metabolites of chemical A cause liver toxicity, and chemical B potentiates that liver toxicity by enhancing the metabolism of A. Then the interaction, the influence of B on A's toxicity, should be included. Even if the primary toxicity of B, the interacting chemical, is different from the toxicity of concern addressed by the index (e.g., chemical B causes kidney lesions), B is included because it influences the toxicity addressed by HI_{INT}. Contrast this procedure to the additivity-based HI (Section 4.2), where only toxicologically similar chemicals are included. The consequence is that an interaction-based HI can include more types of chemicals than would the additivity-based HI.

The inclusion of interacting chemicals that do not cause the toxicity of concern in the calculation does not cause any difficulties. In the above example, if chemical B does not cause liver toxicity, then its HQ is zero. Chemical B then only enters the calculation through its influence on the toxicity of chemical A.

An improved HI_{INT} would result if the default functions, f and g, could be replaced by empirically derived models that reflect the dose-dependence of the interaction. Such information is rare, and, although encouraged, is not required.

4.3.3. 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 originally 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 A on toxicity of chemical B can be different than for chemical B on toxicity of chemical A.

The methods for modifying the HI 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 interaction studies are completed and more interaction mechanisms and modes of interaction are understood, these algorithms will be revised.

4.4. RELATIVE POTENCY FACTORS

4.4.1. Introduction

The toxicity (i.e., magnitude of toxic effect) of a chemical mixture is best determined by direct toxicologic evaluation. When such studies are available for all of a mixture's component chemicals, they may be used to develop a hazard index (see Section 4.2). Because of the temporal and monetary constraints imposed by epidemiologic studies or direct toxicologic evaluation of the components or the mixture as a whole, other approaches that rely more heavily on scientific judgment 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 toxicity inferences.

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 toxicologic dose-response data for at least one component of the mixture (referred to as the index compound) and scientific judgment 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 because of data limitations; RPF application may also be limited to a specific route of exposure or exposure duration. The toxicity of the related compounds is predicted from the index compound by scaling the exposure level of each compound by its toxicity relative to the index compound. This scaling factor or proportionality constant is based on an evaluation of the results of a (usually) small set of toxicologic 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, i.e., it requires ten times the exposure to cause the same toxicity, then 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 negligible toxicity, all of their RPFs could be assigned a value of 0.

In the RPF approach, an exposure equivalent to the index compound is the product of the measured concentration of the mixture component and the RPF. These dose equivalents are summed to express the mixture exposure in terms of an equivalent exposure to the index compound; risk can be quantified by comparing the mixture's equivalent dose in terms of the index compound to the dose-response assessment of the index compound. This estimate of equivalent index compound exposure should be considered an interim and approximate decision-making tool. The RPFs must be defined as to the scope of toxicologic effects that are covered, and the degree of similarity in chemical structure and mode of action that can be inferred from the summation of the adjusted exposure levels. (Mode of action refers to a continuum that describes the key events and processes starting from the point of toxicant-cell interaction and leading to the onset of a health endpoint). In general, the mixture concentration expressed in terms of the index compound for n compounds is,

$$C_m = \sum_{k=1}^{n} C_k * RPF_k \qquad (4\text{-}18)$$

where

C_m = mixture concentration expressed as index compound,

C_1 = concentration of the index compound in mixture,

C_k = concentration of the kth mixture component, and

RPF_k = proportionality constant for toxicity of the kth mixture component relative to the toxicity of the index compound.

Clearly, $RPF_1=1$, as k=1 indicates the index chemical.

To date, the Agency has developed three examples of RPFs that estimate the toxicity of a mixture of related compounds. Each of these examples has been developed as an interim measure pending the development of more case-specific data. The three classes of compounds for which relative potency approaches have been examined by EPA are the dioxins, the polychlorinated biphenyls (PCBs), and the polycyclic aromatic hydrocarbons (PAHs). 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.

4.4.1.1. *Dioxins*

In March of 1989, 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 also were discussed and adopted internationally (Mukerjee and Cleverly, 1987; NATO/CCMS, 1988). In addition to describing the regulatory need and the process of achieving scientific consensus, the 1989 EPA document cautiously recommended comparing available toxicologic 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 on the basis of such data as information regarding human carcinogenicity, carcinogenic potency based on animal studies, reproductive effects data, in vitro test data, and structure-activity relations. Van Leeuwen (1997) and van den Berg et al. (1998) identified each comparison of toxicity from an individual experiment as a relative potency value, or REP. The term TEF was reserved for consensus toxicity estimates where a single TEF is 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 toxicologic 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 mode 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 implies the existence of a larger data set upon which to base toxicologic comparisons 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, 1991a). It must be noted that a key assumption for the dioxins was that a single TEF could apply to all toxic endpoints, all routes of exposure, and for all exposure durations. 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 mode 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 4.4.1.2.

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 TEFs that reflected a consensus judgment of the expert panel. For REPs from 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 nonadditive interactions as well as antagonism and potentiation (e.g., Davis and Safe, 1989; Safe, 1994; Birnbaum et al., 1985). This TEF approach and the TEF values developed have been adapted and presented in the draft dioxin reassessment (U.S. EPA, 2000b).

Interestingly, the WHO expert panel (Van den Berg et al., 1998) extended the TEF approach for this group of compounds to three classes of nonmammalian chordates, developing consensus TEFs 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.

4.4.1.2. *PCBs*

The Workshop Report on Toxicity Equivalency Factors for Polychlorinated Biphenyl Congeners (U.S. EPA, 1991a) reported that certain groups of PCBs appear to share a common mode of action 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 that report and others (e.g., Ahlborg et al., 1994) that 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, 1991a). TEFs were proposed only for some members of the class, and the TEFs proposed were considered applicable only to the health endpoint of cancer through the common mode of action shared with the dioxins.

When assessing PCB mixtures, it is important to recognize that both dioxin-like and non-dioxin-like-like modes of action contribute to overall PCB toxicity (Safe, 1994; McFarland and Clarke, 1989; Birnbaum and DeVito, 1995). 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 non-dioxin-like-like modes of action.

Because PCB cause cancer by both dioxin-like and non-dioxin-like modes of action, both dioxin-like and non-dioxin-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 non-dioxin-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 non-dioxin-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. (It should be noted that the cancer slope factor for PCBs in U.S. EPA 1996a was developed at a time when the concentration of the dioxin-like PCB congeners in the tested mixture had not been reported. This information has since become available [Cogliano, 1998] and EPA is revising the procedure by which dioxin equivalence is estimated.)

4.4.1.3. *PAHs*

The Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) (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) PAHs 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 PAHs were compared with those of B[a]P to estimate cancer potency. The approach assumed that the B2 PAHs had the same cancer slope factor as B[a]P. The ability of these non-B[a]P B2 PAHs 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 PAHs, 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 PAHs. To estimate cancer risk for the B2 PAHs, the cancer slope factor for B[a]P was multiplied by the estimated order of potency and by the concentration of the specific PAH.

4.4.2. Procedures for Developing a Relative Potency Factor (RPF) Approach

TEFs for dioxins were the first RPFs developed and reflect a chemical group with the broadest database examined to date and an apparent uniform mode of action. The criteria for developing TEFs are more rigorous than can be met by most classes of chemicals. However, TEFs provide the background for the procedures for general 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 4-5). The RPF is viewed more broadly than the TEF and can be formulated by the following procedures. Typically RPFs will be developed by a cross-disciplinary group of scientists to address specific regulatory needs.

4.4.2.1. *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. The RPF approach is considered to be an interim method of dose-response assessment and its application may be more uncertain than other methods.

4.4.2.2. *Initiate the RPF Process*

When developing an RPF, both the appropriate data and the relevant scientific expertise needed to evaluate the data must be assembled. The minimum data needed for development of an RPF approach include: (1) a known or suspected common mode of action shared by the class of compounds; (2) a quantitative dose-response assessment for the index compound; and (3) pertinent scientific data that allow the components to be meaningfully compared to the index compound in terms of relative toxicity. The relevant toxicologic data for the individual components may include short-term or chronic in vivo assays, in vitro assays, and quantitative structure-activity relationship data. Because the RPF approach relies heavily on the judgment of scientific data, it may be important to assemble a cross-disciplinary group composed of scientists who have established expertise for the given chemical class or understand the relevance of the various toxicologic assays to human health risks. This group can assemble, interpret, and

Table 4-5. Differences Between TEFs and RPFs	
TEFs	**RPFs**
Specific type of RPF	Generalized case
Apply to all heath endpoints	May be limited to specific health endpoints
Apply to all exposure routes	May be limited to specific routes
Apply to all exposure durations	May be limited to a specific exposure duration
Imply more abundant data and greater certainty about mode of action	May be based on lower quality/fewer data and less certainty about the mode of action

integrate the relevant scientific data and may know of ongoing research activities that could be brought to bear on the process. This scientific group may also be useful in the evaluation and limitations of the final product(s) of the approach.

4.4.2.3. *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 the understanding of the common mode of action leading to the observed toxicologic effects, the chemical similarity of the compounds, and the identification of the spectrum of toxicologic impacts shared by the class. The compounds should also be known to occur as mixtures in environmental samples. If exposures to the class compounds are not simultaneous, the RPF approach may still be valid. Sequential exposures could result in overlapping internal doses, or overlapping effects because of persistence of single-chemical effects. In those cases, dose addition could be an appropriate approximate characterization of the mixture exposure, and so the RPF approach may be adequate for the mixture risk assessment. Example applications have not been located in the literature, so each case must be considered on its own merits. Exposures to different chemicals in the class that are widely separated in time, however, may be better characterized by separate assessments that treat the chemicals independently.

While 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, fraction of unidentified material in typical environmental mixtures, purity of the individual compounds when assayed, the costs related to chemical analysis, the identification of toxicologic impacts not shared by the class of compounds,

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. The pertinent data include dose-response data over a relevant range of doses.

4.4.2.4. ***Develop the RPF***

4.4.2.4.1. ***Select the index compound.*** All RPFs will be based on comparisons of toxicity with that of an index compound. It is preferable to have a single index compound for the RPF approach to promote consistency of application and interpretation. The index compound should have a quantitative dose-response assessment of acceptable scientific quality. It is presumed that typically the index chemical will be the best studied member of the class and have the largest body of acceptable scientific data. The pertinent data include exposure data for the routes and duration 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 is most representative of the class and has the most extensive and best quality database. Once the set of toxicologic assays has been chosen for determining the RPF values, the selection of the index compound will not impact the calculation of the equivalent mixture exposure level because the relative magnitudes of the RPFs compared to each other will be unchanged. The index compound selection does change which dose-response function will be used in interpreting the equivalent mixture exposure in terms of health risk. Consequently, when there are multiple candidates for index chemical, the uncertainty or range in the resulting mixture risk estimate should reflect the differences in the index chemical dose-response function, both regarding overall quality as well as relevance to the exposure conditions being assessed. For example, when exposure conditions represent more than one route, it may be more appropriate to select a different index chemical for each exposure route, i.e., one with the best dose-response data for that route. Because 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 compounds not selected could be used to assess confidence in the approach in at least a limited manner.

4.4.2.4.2. ***Describe the scientific basis for the RPF.*** The scientific criteria for RPF development need to be clearly stated. The known or suspected common mode of action shared by members of the class of compounds should be described. If the toxicologic assays used to develop the RPFs were ranked, the justification for the ranking and its application should be described. For example, some RPFs 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, and thus, the results of these in vivo studies may be weighted more heavily than in vitro test data (see discussion of minimum criteria for RPF developed in Section 4.4.2.2).

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 along with the reasons for the decisions described.

4.4.2.4.3. ***Assign RPF.*** A description of the approach used to determine the RPF values 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 convey better the uncertainty to potential end users in the three examples presented in Section 4.4.1, RPFs were assigned only as order of magnitude estimates. Clearly, the certainty or precision of the approach should not be overstated.

When two or more assays are available to compare the toxicity of a class of compounds with the index compound, multiple assay results could be used. For example, three RPF values could be derived for one compound by using data from three different studies. The body of scientific data used to determine an RPF for a specific member of a chemical class may be portrayed as a range or a distribution. The resulting RPF range or distribution would still require justification, including interpretation and impact of the individual toxicologic studies from which the RPFs were developed.

4.4.2.5. ***Characterize Uncertainty***

The strongest recommendation expressed in the U.S. EPA Chemical Mixture Guidelines (U.S. EPA, 1986) (Appendix A) is to describe the uncertainties in risk assessment. This step is crucial to proper interpretation of the RPF approach and the resulting mixture risk assessment. The areas of uncertainty described below are considered to be a minimum of what should be discussed. Other uncertainties that arise during the application to a specific mixture should also be addressed.

4.4.2.5.1. ***Define the health endpoints and exposure routes covered and not covered by the approach.*** In this step the scientific support for including or excluding the various endpoints and routes in the RPF approach should be carefully described. The applications of scientific judgment in the process of RPF development should be identified and described.

For the widest application, a data set encompassing a variety of animal species, exposure durations, health endpoints, and exposure routes is needed. In the best cases it can be stated with some confidence whether the effect on which the RPF is based is the most sensitive; the full spectrum of health impacts may also be known. For those classes of compounds with less than complete toxicologic 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. A risk assessment applying the RPF 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.

4.4.2.5.2. ***Determine the consistency within the group of compounds considered.*** If multiple health endpoints, exposure durations, or multiple exposure routes are covered by the RPF, the issue of consistency across routes, durations, and endpoints should be addressed. 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 indicate uncertainty surrounding the common mode of action or uncertainty about the relationships between the class members and the index compound. Uncertainty of no more than two 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.

4.4.2.5.3. ***Assess mode of action.*** It is necessary to describe the mode of action of the class of compounds underlying the health effects for which the RPF was developed. A common mode of action for the class is the basis for the assumption of dose additivity. However, in some cases the class may be linked by common effect with only suggestive or indirect information concerning the underlying mode of action. The description of the RPF must answer the question, "to what degree do the scientific data support the assumption of a common mode of action?"

4.4.2.5.4. ***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 studies that examine simple mixtures of the chemical class. If

these studies support the assumption of dose additivity, they increase the confidence in applications of the approach. If they indicate that there are synergistic or antagonistic interactions that are not being considered, then the final answer based on the RPFs may be unrealistic, and so a different approach such as the interaction-based hazard index should be considered. Interactions noted only at high exposures, however, should be viewed cautiously because they may not occur at lower environmental exposures. Pharmacokinetic differences among the class of compounds should be identified because differences in the pharmacokinetics across species could substantially change RPFs developed from nonhuman data.

Chemical mixtures may exhibit dose additivity over certain combinations of dose ranges for the individual chemicals but may not exhibit dose additivity over others. A methodology for detecting regions of additivity and/or departure from additivity has been proposed (Gennings et al., 1997; Gennings, 1995). A key feature of the methodology is that it enables generation of experimental designs that are practical in size, being based only on dose-response data for each component in the mixture.

4.4.2.5.5. ***Examine the issues related to application of the RPF.*** The purpose of this step is to allow the developers of an RPF approach to describe their concerns that relate to application of the RPF. The concerns of those that develop an RPF approach are viewed as related, but distinct, from those of the end users that apply it. Their concerns may pertain to the overall confidence in the application when most of the toxicity is based on a subset of components with weaker data (e.g., this could be related to lower confidence in the common mode of action). They may also have concerns about confidence for certain exposure routes or endpoints. The developers of the RPF should note any differences in pharmacokinetics across the class. When PBPK models are not available and external exposure levels must be used, the assumption for simultaneous exposures is that the pharmacokinetics are similar across the class or that a rough proportionality exists between the external exposure and the tissue dose. However, when the exposures to the class compounds are sequential, then differences in the pharmacokinetics could result in overlap of internal doses from the separate exposures. Such information should be described for consideration by the end user of the RPF. (See previous discussion in Section 4.4.2.3.)

4.4.2.6. ***Evaluation of the RPF***

The RPF approach should undergo scientific peer review. The review should evaluate the scientific judgments employed in each step of RPF development as well as issues related to RPF application. The review should assess the following:

- judgment that a common mode of action is shared by members of class,
- assignment of class membership,

- scientific data supporting each of the RPFs,
- consistency of the RPFs across the class for multiple routes or endpoints,
- the appropriateness of specific limitations pertaining to exposure route or target organ, and
- application issues.

4.4.2.7. *Research Needs*

The RPF approach does not use direct toxicity data on every member of the chemical class; it is considered to be an interim method, to be replaced by better approaches when the required data are available. The method most often recommended to replace the RPF (or TEF) approach is a component-based assessment using actual dose-response data on each chemical in the class. The resulting approach could be a Hazard Index, which is also based on dose addition, or a response addition estimate of probabilistic risk, as is common for cancer risk assessments (Hertzberg et al., 1999; U.S. EPA, 1989).

4.4.3. Risk Characterization Using RPFs

4.4.3.1. *TEF-Based Assessments*

When a mixture exposure is completely described by 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.

4.4.3.2. *General RPF-Based Assessments*

When all chemical class members are assigned single RPFs that represent all effects, exposure routes, and durations, 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 deemed necessary for one or more mixture components, e.g., for different exposure routes or toxic effects, a separate mixture assessment should be developed 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.

Quantitative mixture risk assessments based on RPFs, even those that satisfy the requirements for TEFs, are weaker than those assessments based on direct toxicity data. The uncertainty description is then a key part of the risk characterization. The discussion of uncertainties and overall confidence in the risk assessment should characterize the contribution

of the index chemical to the total predicted equivalent exposure estimate. Similarly, the fractions of predicted equivalent exposure that result from components that exhibit direct evidence of human health effects and from the components for which direct toxicity data are available should also be quantified. When most of the mixture risk is based on inferred toxicity (e.g., 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. (This is particularly important if there is a large disparity between the index compound and other members of the class with respect to the quantity, quality, and pertinence of the data set). Confidence in this approach for a given chemical class must be characterized in the context of 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.

The RPF should be carefully defined as to its underlying limitations, including the notation that the value obtained is an estimate of exposure, and might not be extended to quantitative risk assessment. Analysts applying the RPF should also evaluate evidence for dose and route extrapolations, including the relevance of toxicologic 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 such RPFs.

When the data are judged inadequate to use the above RPF procedures, an approach could be adopted where all compounds in the class are assumed to be as toxic as the index chemical. Adoption of this 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.

For some mixtures there are analytical limitations. Some members of the class that are present in the chemical mixture may not be identified, but their presence may be inferred from measures of mass balance. The procedure for including these compounds in the risk assessment should be clearly defined.

4.4.4. Hypothetical Example of RPF Approach

The application of RPFs to the estimation of risk from a mixture of compounds that exert the same toxic effect by similar mode of action can be demonstrated by the following hypothetical 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, an RPF approach is judged to be appropriate for combining risk of cholinesterase inhibition from this group of chemicals.

After determining that there is a regulating need, the first step in developing a set of RPFs for a group of chemicals is to evaluate the data available for each and identify the chemical whose data set appears to be the most extensive and that best describes the toxicologic propensity of the chemicals in question. In Table 4-6, the information on the five chemicals in question is summarized. From this data set, 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

Table 4-6. Characterization of the toxicologic properties of five cholinesterase-inhibiting chemicals

Chemical	Study ED_{10} (mg/kg/day)	Test species	Duration of critical study	Data set 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.

number of species, and clearly relating the effects in test species to humans. The data sets for the other compounds were not as extensive or well documented. In one case, only a few poor-quality dose-response studies were available, although they provided an acceptable basis for calculating an RPF. (Despite their limitations, these studies were judged to be useful for the development of the RPF. They were judged to provide a better basis for assessing risk than the use of other simple assumptions such as the toxicity of these compounds is equal to the index compound, i.e., RPF = 1, or their toxicity is negligible, i.e., RPF = 0.) The data sets for each compound must next be evaluated to determine the critical study and effect levels 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 can be done by dividing the ED_{10} (U.S. EPA, 1996e) for the critical study of chlorophos by the ED_{10} derived from the critical study for each compound. The results of this calculation for the example data are presented in Table 4-7.

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 4-7. However, the information is compound specific and cannot be directly combined. Using the calculated RPFs, the exposures for each of the chemicals are

Table 4-7. Relative potency factors and equivalent exposures for five cholinesterase-inhibiting chemicals

Chemical	Study ED_{10} (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	0.3
Ethaphos	0.0 6	5	0.05	0.3
Deltaphos	0.1 5	2	0.15	0.3
Total				0.95
Percentage of RPF - predicted toxicity associated with the index compound			32 %	

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 and issues are evident in this example:

- The first is that the points of departure (here, ED_{10}) 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, all the RPFs should be calculated using data from a single species to the extent possible.
- The durations 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 (or the exposures) are not cumulative over time. Where there is evidence that effects are cumulative, studies used for calculating RPFs should be of similar duration.
- If the risk manager is interested in potential effects of exposures to these compounds other than cholinesterase inhibition (e.g., carcinogenicity, nephrotoxicity, and hepatotoxicity), then a separate assessment needs to be developed.

4.5. RESPONSE ADDITION

4.5.1. Background

Response addition is usually applied when the mixture components are assumed to be toxicologically independent (see Section 4.1.1.2) and when exposure to one chemical has no influence on the likelihood or extent of toxicity caused by a second chemical. Such a condition is highly dependent on the exposure levels and may also depend on the route of exposure. The following discussion assumes that information supporting toxicological independence is available for the exposure scenario being assessed or that the extrapolation is justified.

When two chemicals cause different kinds of toxicity, or induce effects in different organs, they may be candidates for response addition, where the responses as probabilities of toxic effects are combined. There are two applications of response addition with somewhat

different calculations: likelihood of an individual showing toxic effects (see Section 4.5.2), and the proportion of a population showing toxic effects (see Section 4.5.3). Because the population aspect is different from the physiological independence discussed earlier, both issues need to be addressed when assessing population risk. A key concept with both applications is functional independence: whether exposure to chemical A has any influence on the toxicity produced by exposure to chemical B.

For joint exposures to one individual, the concern is whether the two chemicals cause toxicity by different processes, such as different target organs or different modes of action in the same organ. The response measure must be the probability of a specific toxic effect. When applied to an individual, the assumption for response addition is that the two chemicals produce toxicity independently.

For joint exposures to a population, a different issue is whether the chemicals cause toxicity to the same proportion of the population (U.S. EPA, 1990). The tolerance distribution for chemical A shows the proportion of individuals responding as the exposure level of A increases. For example, consider the simplest mixture of only two chemicals. If the two chemicals' tolerance distributions are perfectly correlated, then the ordering of individual sensitivities is the same for both chemicals, i.e., the individual most sensitive to chemical A is also most sensitive to chemical B. The most toxic chemical then produces the toxic response first in any of the individuals exposed. Although the severity of the toxicity may be exacerbated by the second (less toxic) chemical, the number of individuals responding is determined only by the most toxic chemical. This issue and the limitations in addressing population risk based on correlations of tolerance distributions are discussed more fully in Section 4.5.3.

Few empirical studies have evaluated response addition in any depth, but the concepts they address suggest possible research directions. Of the few studies at low exposure levels that have modeled joint toxic effects as probabilities, most consider cancer, obviously influenced by the much wider availability of response data for cancer when compared to other kinds of toxicity. In a conceptual investigation of the performance of both the multistage model and the two-stage clonal expansion model for carcinogenesis, assuming an experiment using a balanced 2×2 design with 50 animals per dose group and a strong synergistic interaction, NRC (1988, p. 193) concluded that if the exposure to one or both agents is lowered by two orders of magnitude from the experimental doses, the assumption of response additivity "is reasonably good" in predicting the true mixture response.

Gibb and Chen (1986) also considered implications of the multistage model. They showed that at low doses, the risks are additive for carcinogens acting on the same stage, whereas the hazard functions are multiplied when calculating risks for carcinogens acting on different stages. Brown and Chu (1988) 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. . . ."

The primary requirements for response addition are the availability of data on population fraction or percent response, and the assumption of functional independence. The other major assumptions often used by EPA, the assumption of no threshold dose, low-dose linearity, and interspecies scaling by body allometry, are not relevant to the premise of independence, although they certainly may play a role in estimating the magnitude of an interaction. To simplify the discussion, the following will address the case of the binary mixture, i.e., chemicals A and B.

4.5.2. Individual Toxicity

When an individual is exposed to two chemicals, A and B, there is the potential for A to affect the toxicity of B, and vice versa. When the toxicity of each chemical is totally described by its own exposure level, the two chemicals are said to be independent. The interpretation is that chemicals A and B may cause some toxicity in the individual, but the presence of A (and its toxicity) has no influence on the toxicity of B, and similarly, B has no influence on the toxicity of A. In this context, the two concepts of functional (or physiological) independence and statistical independence are consistent.

In the case where the toxicity of the two chemicals is the same type, say abnormal liver function, then the estimated mixture response may be expressed in terms of general abnormal liver function. At high doses, there may be physiological interactions between two different toxicities. At low doses, especially when the affected tissues are physically separated and only a small fraction of the tissue is damaged, the assumption of independence may hold. As shown by Feron et al. (1995), toxicity within the same target organ but of different modes of action may indicate independent processes (response addition) or similar processes (dose addition), or even some intermediate characterization.

When the component effects are of minor severity, independence for different modes of action seems plausible. One must be cautious about assuming independence in the same target organ and then concluding that two minor effects are minor in the aggregate. If an organ is compromised twice, its function may be worse than from exposure to either chemical alone. When information is lacking on joint effects in the same organ, a conservative approach is to assume dose addition.

Independence in quantitative risk assessment is often used when determining the probability of an adverse effect from exposure to multiple chemicals. If the toxicity measure is

the probability of an individual incurring toxic damage, then independence can be expressed by the probabilistic definition:

$$p_m = 1 - (1 - p_1) * (1 - p_2) \tag{4-19}$$

where p_m is the expected response from exposure to the mixture, and p_1 and p_2 are the responses from exposure to chemicals A and B, respectively. This equation says that the response to the mixture (caused by chemical A or B) is 1 minus the probability of not responding to either chemical. Expanding the right-hand side and including the exposure levels d_1 and d_2 for chemicals A and B, respectively, one obtains:

$$p_m(d_1, d_2) = p_1(d_1) + p_2(d_2) - p_1(d_1) * p_2(d_2) \tag{4-20}$$

In general, the formula is:

$$p_m = 1 - (1 - p_1) * (1 - p_2) * (1 - p_3) * \ldots \tag{4-21}$$

or in more compact notation:

$$p_m = 1 - \prod_{i=1}^{n} (1 - p_i) \tag{4-22}$$

The product on the right-hand side is the probability under independence of not responding to any of the chemicals. The second form of the formula (Equation 4-22) then clearly shows that the probability of responding to the mixture is just 1 minus the probability of not responding to any of the component chemicals.

Example. Applying this to a large number of chemicals (40), each posing a very small risk (3×10^{-5}),

No. of chemicals	40
Single-chemical risk	3×10^{-5}
Mixture risk	$1 - (1 - 3 \times 10^{-5})^{40} = 1 \times 10^{-3}$

4.5.3. Population Toxicity

The dose-response assessment is different when considering the entire population of exposed individuals. The risk is often then presented as the percent responding in the population. Independence is not a matter of physiological interactions within an individual, but is based on the correlation of tolerances for the two chemicals (see U.S. EPA, 1990, for an extended

discussion). The tolerance distribution for any given chemical is the proportion of people responding as the exposure level of that chemical increases.

For exposure to two chemicals, A and B, the ordering of the individual sensitivities to chemical A is the same as the ordering for chemical B, then the tolerances for the two chemicals are perfectly correlated (r = 1), and the most toxic chemical will elicit the response first:

$$p_m = p_2 \quad if\ r = 1\ and\ p_1 < p_2 \tag{4-23}$$

If, on the other hand, the individual least sensitive to chemical A is most sensitive to chemical B, and so on throughout the range of sensitivity, then the chemicals have perfect negative correlation (r = -1) of tolerances, and the mixture response is:

$$p_m = min(p_1 + p_2, 1) \quad if\ r = -1 \tag{4-24}$$

When the correlation is zero (r = 0), i.e., the ordering of the individuals showing toxic effects from chemical A has no apparent relationship with the individuals showing toxic effects from chemical B, then the two chemicals are said to act independently on the population. We then have the familiar model for statistical independence:

$$p_m = p_1 + p_2 - (p_1 p_2) \quad if\ r = 0 \tag{4-25}$$

Equation 4-25 is the same model described above for toxicologic independence in a single exposed individual.

The response-addition formula for populations has limited use in risk assessment. First, it is more complicated than the formula for the individual, because the tolerance correlation can be any value from -1 to +1, and so requires more detailed data on the exposed population of concern. In addition, the concepts of tolerance correlation only work well if there are two chemicals in the mixture. For example, if a mixture has three chemicals, then the correlation of tolerances must consider the three possible pairs of chemicals. No methods have been found for using pairwise tolerance correlations in higher complexity mixtures. Also, some correlation values cannot be applied to three or more chemicals. For example, tolerances of three chemicals cannot all be negatively correlated with each other. The well-studied cases using tolerance correlations are those discussed in this section. Consequently, response addition for populations is not further developed in this guidance document.

4.5.4. Application

Response addition is easily misinterpreted because of the appearance of accuracy and precision given by the use of numbers to represent the risk of toxic effects. In contrast, it is hoped that the HI is less likely to be over interpreted because it only indicates a rough level of concern, not a probability or population count. Errors may arise from improper use of response addition because of a lack of independence. With a mixture of a large number of chemicals, it is particularly easy to overlook the influence of a poor-quality response estimate. Mixture assessments based on response addition must include quality descriptions for each component's response estimate.

For single-chemical responses p_1, p_2, ..., response addition applied to the risk to an individual is often approximated by the simple summation:

$$p_{SUM} = (d_1, d_2, \ldots) = p_1(d_1) + p_2(d_2) + \ldots \tag{4-26}$$

For mixtures of a few chemicals and very small p, this approximation may be acceptable. For mixtures with a large number of component chemicals or chemicals whose response is not small, the full independence formula (Equation 4-21) should be used. For example, with a simple mixture of only 16 chemicals, if each has a response of 0.02, the relative error is 16% (sum in Equation 4-26 gives 0.32, true response from Equation 4-21 is 0.28). Because of the availability of computers, the full formula (Equation 4-21) is easily implemented and should be used.

The other concern with a large number of chemicals in the mixture is that one poorly studied chemical may dominate the response estimate. An excessive response estimate could arise from improper statistical analysis or toxicological procedures employing highly sensitive animal species. Similar factors could also lead to response estimates that are too low, often caused by lack of statistical power in the study design. In all cases, the risk characterization should highlight any chemicals whose supporting information is poor, and should attempt to characterize the numerical uncertainty caused by the poor information. For example, if only one chemical has a highly uncertain response estimate, the mixture assessment can be calculated with and without the suspect chemical.

For minor toxic effects, the different effects are unlikely to interact, so the response addition formula (Equation 4-21) is probably adequate. One mixture response could then be estimated for all renal toxicity, with another estimated for all hepatic toxicity. The mixture assessment could then result in several separate response addition estimates, one per effect or target organ. For levels causing moderate toxicity, there is insufficient information to allow predictions of the likelihood of physiological interactions between affected target organs. For high-exposure estimates, additive formulas are not generally recommended because of the higher

likelihood of toxicologic interactions (e.g., in the same tissue) among component chemicals in the mixture as well as physiological interactions among the various affected target organs.

For low exposure levels, e.g., near the individual chemical NOAELs from well-designed studies, toxicologically dissimilar chemicals are not generally expected to interact toxicologically or physiologically, and can be assumed to be functionally independent. For the special case where all component chemicals have RfDs or RfCs and the exposure levels of dissimilar components are well below their respective RfDs or RfCs, the risk of toxicity can usually be assumed to be negligible.

Example. Consider an oral exposure to three toxicologically independent chemicals, each close to but below its RfD. The following calculations result:

Chemical	Exposure	RfD	Risk
A	13	16	0
B	7	8	0
C	22	24	0

Mixture risk = 0

In this example, 0 is used to denote a risk that is either subthreshold (a true zero risk) or small enough to be generally considered virtually safe. In general, this kind of rough evaluation should be limited to mixtures with a small number of chemical components. When the number of chemicals in the mixture is large, even when all individual exposures are below their RfDs, the toxicity data should be carefully examined to ensure that all effects and modes of action are being considered when deciding functional independence. As the information becomes more uncertain, such as with poor-quality RfDs or exposure estimates, any conclusion of negligible risk is similarly uncertain and consideration should be given to obtaining better information.

4.5.5. Use of Upper Bound Response Estimates

The practice of assessing cancer risk for a mixture has usually involved applying response addition to the lifetime excess cancer risk values available for the individual chemicals (U.S. EPA, 1986). The common values generated by EPA are those available on the IRIS database. Currently, most of the IRIS values for carcinogenic potency are for single chemicals and are considered plausible upper bounds to the actual lifetime excess cancer risk. Use of such values raises the concern that applying response addition to upper bounds will lead to unreasonably high estimates of the actual upper bound on mixture risk. The available studies, summarized below, suggest that for most mixtures of a few components, the risk estimates are not overly conservative.

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 error from using the simple sum of upper bounds occurred when all chemicals were roughly equal contributors to the mixture risk. Their proposed method for the upper 95% confidence bound of a two-chemical mixture reduced the conservatism, but only slightly. Their mixture upper bound was 4.3×10^{-7}, whereas the simple sum of the component upper bounds was 4.9×10^{-7}.

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. Thus, although the confidence attached to the mixture bound may exceed the confidence levels for the component chemicals, the actual mixture risk estimate (i.e., its magnitude) is not excessively high. Cogliano concludes that simple sums of upper bounds are a good approximation of the overall risk and can be adjusted downward (e.g., by dividing by 2) to give a more plausible upper bound, or even a central estimate of overall risk.

These two measures of overconservatism, the estimate and the confidence level, are also discussed in Cullen (1994). In contrast to Cogliano's results for sums of upper bounds, Cullen showed substantial overconservatism for products of upper bounds.

4.5.6. Qualitative Judgments of Interaction Potential

Response addition may work well for many mixtures at very low doses with components affecting different target organs. Other mixtures, even at low doses, may show evidence of toxicologic interaction. In the example method described in this section, the key assumption is that interactions in those mixtures can be adequately represented as departures from response addition. The method follows an obvious approach: to begin with the response addition formula, and then modify its estimate to reflect the interaction results. Although several studies describe toxicologic interaction as a departure from response addition (e.g., changes from the predicted LD_{50}), 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.

Carcinogen interactions are the basis for the example method that follows (Section 4.5.6.1, Equation 4-27). The modeling of carcinogenic interactions is in an early stage of development. Consequently, the following method is not currently recommended as a quantitative method for adjusting the mixture risk estimate. It should be considered as a possible approach to a qualitative description of the interactions in a mixture. Because of the dominance of binary mixtures in interaction studies, only pairwise interactions are included in the example method. A tacit assumption is that higher order interactions are relatively minor compared to binary interactions.

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 workers with exposure to cigarette smoke and/or asbestos. Hammond et al. (1979) noted that in comparison with the lung cancer death rates for nonsmokers 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). Under response addition, where the two exposures are assumed to be independent causes of lung cancer, the expected response from the joint exposure was 169.7 lung cancer deaths per 100,000 man-years exposure, yet the observed response was 601.6 per 100,000. Note that the exposure levels in this example are much higher than usual ambient environmental exposures, so other instances of synergism between carcinogenic chemicals may be much less pronounced.

This synergism between asbestos and smoking is commonly described as an example of a multiplicative interaction (Mukerjee and Stara, 1981). This term is used because when the numerators in the single substance death rates are multiplied, the product is roughly equal to the numerator in the death rate for the combination (i.e., 5.17×10.85 is roughly equal to 53.24). The risks, however, are not multiplied, and there seems to be no biological process that can motivate such a multiplication of death rate numerators. Similarly, Kodell and Pounds (1991) note that the "multiplicative model of relative risk does not have a corresponding null model in

pharmacology/toxicology studies." As discussed by Greenland and Rothman (1998), there are several definitions of interaction used in toxicology, statistics, and epidemiology, and their interpretations vary by use as well as by the scale of the effect measure. This variety of definitions and their comparative analyses is beyond the scope of this document, but should be addressed by future efforts.

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 database on carcinogen interactions, and in particular on promoters, is not sufficient for recommending a general 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 papillomas or on shortening of the time to tumor. Accordingly, in the absence of an adequate database, 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 database is available in the scientific literature, only qualitative approaches are recommended. In the example method described below for estimating carcinogenic risk of mixtures (Woo et al., 1995b), qualitative judgments of the interaction potential are used to modify a relative ranking of the mixture based on carcinogenic risk.

4.5.6.1. *Use of Interaction Data on Carcinogens*

For known or suspected human carcinogens, past practice at 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 Carcinogen Risk Assessment (U.S. EPA, 1996a) 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 observed 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 observed 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 noncarcinogens contributing to the overall carcinogenic process of the mixture.

There are several databases that 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 risk. 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 that seeks to quantify the potential influence of interactions in carcinogenic mixtures. At this time, the outline is not recommended for quantitative risk assessment but can be further explored as a tool for qualitatively characterizing the potential influence of the interactions.

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 databases 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 databases 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:

$$WR = \frac{1 + (p\ H_{Syn} + q\ H_{Pro})}{1 + (r\ H_{Ant} + s\ H_{Inh})} \tag{4-27}$$

where p, q, r, and s are "hazard-modification effectiveness coefficients" that 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, and

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 judgment and on inspection of the combination effects literature encompassed in their databases. 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 interaction-based HI approach described in Section 4.3. Furthermore, it is not applied to the common unit risk or its counterpart. It is based on a particular literature database and may not generalize to other chemical classes. The potential of this and other approaches to risk assessment that incorporate toxicologic interaction is discussed more fully in Section 2.7, Future Directions.

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EPA/630/R-98/002
September 1986

APPENDIX A

Guidelines for the Health Risk Assessment of Chemical Mixtures

Published on September 24, 1986, Federal Register 51(185):34014-34025

Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Note: This document represents the final guidelines. A number of editorial corrections have been made during conversion and subsequent proofreading to ensure the accuracy of this publication.

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GUIDELINES FOR THE HEALTH RISK ASSESSMENT OF CHEMICAL MIXTURES [FRL-2984-2]

AGENCY: U.S. Environmental Protection Agency (EPA).

ACTION: Final Guidelines for the Health Risk Assessment of Chemical Mixtures.

SUMMARY: The U.S. Environmental Protection Agency is today issuing five guidelines for assessing the health risks of environmental pollutants. These are:

Guidelines for Carcinogen Risk Assessment
Guidelines for Estimating Exposures
Guidelines for Mutagenicity Risk Assessment
Guidelines for the Health Assessment of Suspect Developmental Toxicants
Guidelines for the Health Risk Assessment of Chemical Mixtures

This notice contains the Guidelines for the Health Risk Assessment of Chemical Mixtures; the other guidelines appear elsewhere in today's Federal Register.

The Guidelines for the Health Risk Assessment of Chemical Mixtures (hereafter "Guidelines") are intended to guide Agency analysis of information relating to health effects data on chemical mixtures in line with the policies and procedures established in the statutes administered by the EPA. These Guidelines were developed as part of an interoffice guidelines development program under the auspices of the Office of Health and Environmental Assessment (OHEA) in the Agency's Office of Research and Development. They reflect Agency consideration of public and Science Advisory Board (SAB) comments on the Proposed Guidelines for the Health Risk Assessment of Chemical Mixtures published January 9, 1985 (50 FR 1170).

This publication completes the first round of risk assessment guidelines development. These Guidelines will be revised, and new guidelines will be developed, as appropriate.

EFFECTIVE DATE: The Guidelines will be effective September 24, 1986.

FOR FURTHER INFORMATION CONTACT: Dr. Richard Hertzberg, Waste Management Division, U.S. Environmental Protection Agency, Atlanta Federal Center, 100 Alabama St., SW, Atlanta, GA 30303-3104, TEL: 404-562-8663.

SUPPLEMENTARY INFORMATION: In 1983, the National Academy of Sciences (NAS) published its book entitled *Risk Assessment in the Federal Government: Managing the Process.* In that book, the NAS recommended that Federal regulatory agencies establish "inference guidelines" to ensure consistency and technical quality in risk assessments and to ensure that the risk assessment process was maintained as a scientific effort separate from risk management. A task force within EPA accepted that recommendation and requested that Agency scientists begin to develop such guidelines.

General

The guidelines published today are products of a two-year Agencywide effort, which has included many scientists from the larger scientific community. These guidelines set forth principles and procedures to guide EPA scientists in the conduct of Agency risk assessments, and to inform Agency decision makers and the public about these procedures. In particular, the guidelines emphasize that risk assessments will be conducted on a case-by-case basis, giving full consideration to all relevant scientific information. This case-by-case approach means that Agency experts review the scientific information on each agent and use the most scientifically appropriate interpretation to assess risk. The guidelines also stress that this information will be fully presented in Agency risk assessment documents, and that Agency scientists will identify the strengths and weaknesses of each assessment by describing uncertainties, assumptions, and limitations, as well as the scientific basis and rationale for each assessment.

Finally, the guidelines are formulated in part to bridge gaps in risk assessment methodology and data. By identifying these gaps and the importance of the missing information to the risk assessment process, EPA wishes to encourage research and analysis that will lead to new risk assessment methods and data.

Guidelines for Health Risk Assessment of Chemical Mixtures

Work on the Guidelines for the Health Risk Assessment of Chemical Mixtures began in January 1984. Draft guidelines were developed by Agency work groups composed of expert scientists from throughout the Agency. The drafts were peer-reviewed by expert scientists in the fields of toxicology, pharmacokinetics, and statistics from universities, environmental groups, industry, labor, and other governmental agencies. They were then proposed for public comment in the Federal Register (50 FR 1170). On November 9, 1984, the Administrator directed that Agency offices use the proposed guidelines in performing risk assessments until final guidelines became available.

After the close of the public comment period, Agency staff prepared summaries of the comments, analyses of the major issues presented by the commentators, and preliminary Agency

responses to those comments. These analyses were presented to review panels of the SAB on March 4 and April 22-23, 1985, and to the Executive Committee of the SAB on April 25-26, 1985. The SAB meetings were announced in the Federal Register as follows: February 12, 1985 (50 FR 5811), and April 4, 1985 (50 FR 13420 and 13421).

In a letter to the Administrator dated June 19, 1985, the Executive Committee generally concurred on all five of the guidelines, but recommended certain revisions and requested that any revised guidelines be submitted to the appropriate SAB review panel chairman for review and concurrence on behalf of the Executive Committee. As described in the responses to comments (see Part B: Response to the Public and Science Advisory Board Comments), each guidelines document was revised, where appropriate, consistent with the SAB recommendations, and revised draft guidelines were submitted to the panel chairmen. Revised draft Guidelines for the Health Risk Assessment of Chemical Mixtures were concurred on in a letter dated August 16, 1985. Copies of the letters are available at the Public Information Reference Unit, EPA Headquarters Library, as indicated elsewhere in this notice.

Following this Preamble are two parts: Part A contains the Guidelines and Part B the Response to the Public and Science Advisory Board Comments (a summary of the major public comments, SAB comments, and Agency responses to those comments).

The SAB requested that the Agency develop a technical support document for these Guidelines. The SAB identified the need for this type of document due to the limited knowledge on interactions of chemicals in biological systems. Because of this, the SAB commented that progress in improving risk assessment will be particularly dependent upon progress in the science of interactions.

Agency staff have begun preliminary work on the technical support document and expect it to be completed by early 1987. The Agency is continuing to study the risk assessment issues raised in the guidelines and will revise these Guidelines in line with new information as appropriate.

References, supporting documents, and comments received on the proposed guidelines, as well as copies of the final guidelines, are available for inspection and copying at the Public Information Reference Unit (202-382-5926), EPA Headquarters Library, 401 M Street, SW, Washington, DC, between the hours of 8:00 a.m. and 4:30 p.m.

I certify that these Guidelines are not major rules as defined by Executive Order 12291, because they are nonbinding policy statements and have no direct effect on the regulated

community. Therefore, they will have no effect on costs or prices, and they will have no other significant adverse effects on the economy. These Guidelines were reviewed by the Office of Management and Budget under Executive Order 12291.

Dated: August 22, 1986

Signed by EPA Administrator
Lee M. Thomas

PART A: GUIDELINES FOR THE HEALTH RISK ASSESSMENT OF CHEMICAL MIXTURES

1. INTRODUCTION

The primary purpose of this document is to generate a consistent Agency approach for evaluating data on the chronic and subchronic effects of chemical mixtures. It is a procedural guide that emphasizes broad underlying principles of the various science disciplines (toxicology, pharmacology, statistics) necessary for assessing health risk from chemical mixture exposure. Approaches to be used with respect to the analysis and evaluation of the various data are also discussed.

It is not the intent of these Guidelines 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 these Guidelines.

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 these Guidelines, mixtures will be defined as any combination of two or more chemical substances regardless of source or of spatial or temporal proximity. In some instances, the mixtures are highly complex, consisting of scores of compounds that are generated simultaneously as byproducts 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 class of mixtures consists of compounds, often unrelated chemically or commercially, which are placed in the same area for disposal or storage, eventually come into contact with each other, and are released as a mixture to the environment. The quality and quantity of pertinent information available for risk assessment varies considerably for different mixtures. 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, not all components of the mixture are known, exposure data are uncertain, and toxicologic data on the known components of the mixture are limited. Nonetheless, the Agency may be required to take action because of the number of individuals at potential risk or because of the known toxicologic effects of these compounds that have been identified in the mixture.

The prediction of how specific mixtures of toxicants will interact must be based on an understanding of the mechanisms of such interactions. Most reviews and texts that discuss toxicant interactions attempt to discuss the biological or chemical bases of the interactions (e.g., Klaassen and Doull, 1980; Levine, 1973; Goldstein et al., 1974; NRC, 1980a; Veldstra, 1956; Withey, 1981). Although different authors use somewhat different classification schemes when discussing the ways in which toxicants interact, it generally is recognized that toxicant interactions may occur during any of the toxicologic processes that take place with a single compound: absorption, distribution, metabolism, excretion, and activity at the receptor site(s). Compounds may interact chemically, yielding a new toxic component or causing a change in the biological availability of the existing component. They may also interact by causing different effects at different receptor sites.

Because of the uncertainties inherent in predicting the magnitude and nature of toxicant interactions, the assessment of health risk from chemical mixtures must include a thorough discussion of all assumptions. No single approach is recommended in these Guidelines. Instead, guidance is given for the use of several approaches depending on the nature and quality of the data. Additional mathematical details are presented in Section 4.

In addition to these Guidelines, a supplemental technical support document is being developed which will contain a thorough review of all available information on the toxicity of chemical mixtures and a discussion of research needs.

2. PROPOSED APPROACH

No single approach can be recommended to risk assessments for multiple chemical exposures. Nonetheless, general guidelines can be recommended depending on the type of mixture, the known toxic effects of its components, the availability of toxicity data on the mixture or similar mixtures, the known or anticipated interactions among components of the mixture, and the quality of the exposure data. Given the complexity of this issue and the relative paucity of empirical data from which sound generalizations can be constructed, emphasis must be placed on flexibility, judgment, and a clear articulation of the assumptions and limitations in any risk assessment that is developed. The proposed approach is summarized in Table 1 and Figure 1 and is detailed below. An alphanumeric scheme for ranking the quality of the data used in the risk assessment is given in Table 2.

2.1. DATA AVAILABLE ON THE MIXTURE OF CONCERN

For predicting the effects of subchronic or chronic exposure to mixtures, the preferred approach usually will be to use subchronic or chronic health effects data on the mixture of

Table 1. Risk assessment approach for chemical mixtures

1. Assess the quality of the data on interactions, health effects, and exposure (see Table 2).
   a. If adequate, proceed to Step 2.
   b. If inadequate, proceed to Step 14.
2. Health effects information is available on the chemical mixture of concern.
   a. If yes, proceed to Step 3.
   b. If no, proceed to Step 4.
3. Conduct risk assessment on the mixture of concern based on health effects data on the mixture. Use the same procedures as those for single compounds. Proceed to Step 7 (optional) and Step 12.
4. Health effects information is available on a mixture that is similar to the mixture of concern.
   a. If yes, proceed to Step 5.
   b. If no, proceed to Step 7.
5. Assess the similarity of the mixture on which health effects data are available to the mixture of concern, with emphasis on any differences in components or proportions of components, as well as the effects that such differences would have on biological activity.
   a. If sufficiently similar, proceed to Step 6.
   b. If not sufficiently similar, proceed to Step 7.
6. Conduct risk assessment on the mixture of concern based on health effects data on the similar mixture. Use the same procedures as those for single compounds. Proceed to Step 7 (optional) and Step 12.
7. Compile health effects and exposure information on the components of the mixture.
8. Derive appropriate indices of acceptable exposure and/or risk on the individual components in the mixture. Proceed to Step 9.
9. Assess data on interactions of components in the mixtures.
   a. If sufficient quantitative data are available on the interactions of two or more components in the mixture, proceed to Step 10.
   b. If sufficient quantitative data are not available, use whatever information is available to qualitatively indicate the nature of potential interactions. Proceed to Step 11.
10. Use an appropriate interaction model to combine risk assessments on compounds for which data are adequate, and use an additivity assumption for the remaining compounds. Proceed to Step 11 (optional) and Step 12.
11. Develop a risk assessment based on an additivity approach for all compounds in the mixture. Proceed to Step 12.
12. Compare risk assessments conducted in Steps 5, 8, and 9. Identify and justify the preferred assessment, and quantify uncertainty, if possible. Proceed to Step 13.
13. Develop an integrated summary of the qualitative and quantitative assessments with special emphasis on uncertainties and assumptions. Classify the overall quality of the risk assessment, as indicated in Table 2. Stop.
14. No risk assessment can be conducted because of inadequate data on interactions, health effects, or exposure. Qualitatively assess the nature of any potential hazard and detail the types of additional data necessary to support a risk assessment. Stop.

Note—Several decisions used here, especially those concerning adequacy of data and similarity between two mixtures, are not precisely characterized and will require considerable judgment. See text.

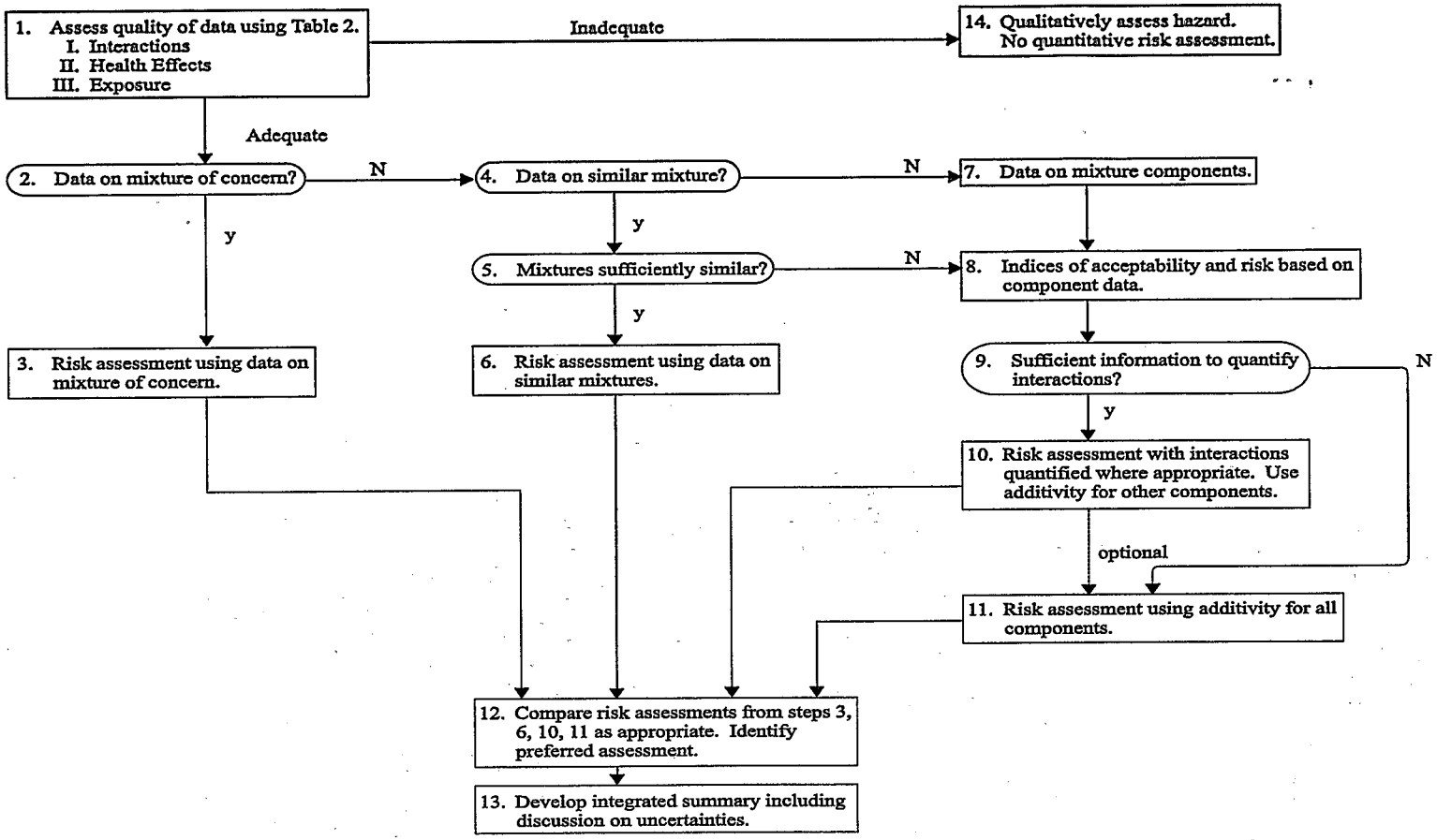

Figure 1. Flow chart of the risk assessment in Table 1. Note that it may be desirable to conduct all three assessments when possible (i.e., using data on the mixture, a similar mixture, or the components) in order to make the fullest use of the available data. See text for further discussion.

Table 2. Classification scheme for the quality of the risk assessment of the mixture[a]

Information on Interactions

I. Assessment is based on data on the mixture of concern.
II. Assessment is based on data on a sufficiently similar mixture.
III. Quantitative interactions of components are well characterized.
IV. The assumption of additivity is justified based on the nature of the health effects and on the number of component compounds.
V. An assumption of additivity cannot be justified, and no quantitative risk assessment can be conducted.

Health Effects Information

A. Full health effects data are available and relatively minor extrapolation is required.
B. 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.
C. 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.
D. Certain important health effects data are lacking and extensive extrapolations are required for route or duration of exposure or for species differences.
E. A lack of health effects information on the mixture and its components in the mixture precludes a quantitative risk assessment.

Exposure Information[b]

1. Monitoring information either alone or in combination with modeling information is sufficient to accurately characterize human exposure to the mixture or its components.
2. Modeling information is sufficient to reasonably characterize human exposure to the mixture or its components.
3. Exposure estimates for some components are lacking, uncertain, or variable. Information on health effects or environmental chemistry suggests that this limitation is not likely to substantially affect the risk assessment.
4. 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.
5. The available exposure information is insufficient for conducting a risk assessment.

[a]See text for discussion of sufficient similarity, adequacy of data, and justification for additivity assumptions.
[b]See the Agency's Guidelines for Estimating Exposures (U.S. EPA, 1986d) for more complete information on performing exposure assessments and evaluating the quality of exposure data.

concern and adopt procedures similar to those used for single compounds, either systemic toxicants or carcinogens (see U.S. EPA, 1986a-c). The risk assessor must recognize, however, 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. Such data are most likely to be available on highly complex mixtures, such as coke oven emissions or diesel exhaust, which are generated in large quantities and associated with or suspected of causing adverse health effects. 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. 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 are diminished.

2.2. DATA AVAILABLE ON SIMILAR MIXTURES

If the risk assessment is based on data from a single 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 also is diminished. This can be offset to some degree if data are available on several mixtures of the same components that have different component ratios which encompass the temporal or spatial differences in composition of the mixture of concern. 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.

If no data are available on the mixtures of concern, but health effects data are available an a similar mixture (i.e., a mixture having the same components but in slightly different ratios, or having several common components but lacking one or more components, or having one or more additional components), 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 additivity. In determining reasonable similarity, consideration should be given to any information on the components that differ or are contained in markedly different proportions between the mixture on which health effects data are available and the mixture of concern. Particular emphasis should be placed on any toxicologic or

pharmacokinetic data on the components or the mixtures which would be useful in assessing the significance of any chemical difference between the similar mixture and the mixtures of concern.

Even if a risk assessment can be made using data on the mixtures of concern or a reasonably similar mixture, it may be desirable to conduct a risk assessment based on toxicity data on the components in the mixture using the procedure outlined in Section 2.B. In the case of a mixture containing carcinogens and toxicants, an approach based on the mixture data alone may not be sufficiently protective in all cases. For example, this approach for a two-component 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 considered by the Agency to be a nonthreshold effect, it may not be prudent to construe the negative results of such a bioassay as indicating the absence of risk at lower doses. 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.

2.3. DATA AVAILABLE ONLY ON MIXTURE COMPONENTS

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 little or no quantitative information is available on the potential interaction among the components, additive models (defined in the next section) are recommended for systemic toxicants. Several studies have demonstrated that dose additive models often predict 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). The problem 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 groups that recommended an approach elected to adopt some type of dose additive model. Nonetheless, as discussed in Section 4, dose additive models are not 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 Federal Register most reasonable additive model should be used.

2.3.1. Systemic Toxicants

For systemic toxicants, the current risk assessment methodology used by the Agency for single compounds most often results in the derivation of an exposure level which is not anticipated to cause significant adverse effects. Depending on the route 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 acceptable daily intakes (ADIs) or reference doses (RfDs), levels associated with various margins of safety (MOS), or acceptable concentrations in various media. For the purpose of this discussion, the term "acceptable level" (AL) will be used to indicate any such criteria or advisories derived by the Agency. Levels of exposure (E) will be estimates obtained following the most current Agency Guidelines for Estimating Exposures (U.S. EPA, 1986d). For such estimates, the "hazard index" (HI) of a mixture based on the assumption of dose addition may be defined as:

$$HI = E_1/AL_1 + E_2/AL_2 + \ldots + E_i/AL_i \qquad (2\text{-}1)$$

where:

E_i = exposure level to the ith toxicant* and AL_i = maximum acceptable level for the ith toxicant.

Since the assumption of dose addition is most properly applied to compounds that induce the same effect by similar modes of action, a separate hazard index should be generated for each end point of concern. Dose addition for dissimilar effects does not have strong scientific support, and, if done, should be justified on a case-by-case basis in terms of biological plausibility.

The assumption of dose addition is most clearly justified when the mechanisms of action of the compounds under consideration are known to be the same. Since the mechanisms of action for most compounds are not well understood, the justification of the assumption of dose addition will often be limited to similarities in pharmacokinetic and toxicologic characteristics. In any event, if a hazard index is generated the quality of the experimental evidence supporting the assumption of dose addition must be clearly articulated.

The hazard index provides a rough measure of likely toxicity and requires cautious interpretation. The hazard index is only a numerical indication of the nearness to acceptable limits of exposure or the degree to which acceptable exposure levels are exceeded. As this index approaches unity, concern for the potential hazard of the mixture increases. If the index exceeds unity, the concern is the same as if an individual chemical exposure exceeded its acceptable level by the same proportion. The hazard index does not define dose-response relationships, and its numerical value should not be construed to be a direct estimate of risk. Nonetheless, if sufficient

data are available to derive individual acceptable levels for a spectrum of effects (e.g., MFO induction, minimal effects in several organs, reproductive effects, and behavioral effects), the hazard index may suggest what types of effects might be expected from the mixture exposure. If the components' variabilities of the acceptable levels are known, or if the acceptable levels are given as ranges (e.g., associated with different margins of safety), then the hazard index should be presented with corresponding estimates of variation or range.

Most studies on systemic toxicity report only descriptions of the effects in each dose group. If dose-response curves are estimated for systemic toxicants, however, dose-additive or response-additive assumptions can be used, with preference given to the most biologically plausible assumption (see Section 4 for the mathematical details).

2.3.2. Carcinogens

For carcinogens, whenever linearity of the individual dose-response curves has been assumed (usually restricted to low doses), the increase in risk P (also called excess or incremental risk), caused by exposure d, is related to carcinogenic potency B, as:

$$P = d\,B \qquad (2\text{-}2)$$

For multiple compounds, this equation may be generalized to:

$$P = \Sigma\, d_i B_i \qquad (2\text{-}3)$$

This equation assumes independence of action by the several carcinogens and is equivalent to the assumption of dose addition as well as to response addition with completely negative correlation of tolerance, as long as $P < 1$ (see Section 4). Analogous to the procedure used in Equation 2-1 for systemic toxicants, an index for n carcinogens can be developed by dividing exposure levels (E) by doses (DR) associated with a set level of risk:

$$HI = E_1/DR_1 + E_2/DR_2 + \ldots + E_n/DR_n \qquad (2\text{-}4)$$

Note that the less linear the dose-response curve is, the less appropriate Equations 2-3 and 2-4 will be, perhaps even at low doses. It should be emphasized that because of the uncertainties in estimating dose-response relationships for single compounds, and the additional uncertainties in combining the individual estimate to assess response from exposure to mixtures, response rates and hazard indices may have merit in comparing risks but should not be regarded as measures of absolute risk.

2.3.3. Interactions

None of the above equations incorporates any form of synergistic or antagonistic interaction. Some types of information, however, may be available that suggest that two or more components in the mixture may interact. Such information must be assessed in terms of both its relevance to subchronic or chronic hazard and its suitability for quantitatively altering the risk assessment.

For example, if chronic or subchronic toxicity or carcinogenicity studies have been conducted that permit a quantitative estimation of interaction for two chemicals, then it may be desirable to consider using equations detailed in Section 4, or modifications of these equations, to treat the two compounds as a single toxicant with greater or lesser potency than would be predicted from additivity. Other components of the mixture, on which no such interaction data are available, could then be separately treated in an additive manner. Before such a procedure is adopted, however, a discussion should be presented of the likelihood that other compounds in the mixture may interfere with the interaction of the two toxicants on which quantitative interaction data are available. If the weight of evidence suggests that interference is likely, then a quantitative alteration of the risk assessment may not be justified. In such cases, the risk assessment may only indicate the likely nature of interactions, either synergistic or antagonistic, and not quantify their magnitudes.

Other types of information, such as those relating to mechanisms of toxicant interaction, or quantitative estimates of interaction between two chemicals derived from acute studies, are even less likely to be of use in the quantitative assessment of long-term health risks. Usually it will be appropriate only to discuss these types of information, indicate the relevance of the information to subchronic or chronic exposure, and indicate, if possible, the nature of potential interactions, without attempting to quantify their magnitudes.

When the interactions are expected to have a minor influence on the mixture's toxicity, the assessment should indicate, when possible, the compounds most responsible for the predicted toxicity. This judgment should be based on predicted toxicity of each component, based on exposure and toxic or carcinogenic potential. This potential alone should not be used as an indicator of the chemicals posing the most hazard.

2.3.4. Uncertainties

For each risk assessment, the uncertainties should be clearly discussed and the overall quality of the risk assessment should be characterized. The scheme outlined in Table 2 should be used to express the degree of confidence in the quality of the data on interaction, health effects, and exposure.

a. Health Effects—In some cases, when health effects data are incomplete, it may be possible to argue by analogy or quantitative structure-activity relationships that the compounds on which no health effects data are available are not likely to significantly affect the toxicity of the mixture. If a risk assessment includes such an argument, the limitations of the approach must be clearly articulated. Since a methodology has not been adopted for estimating an acceptable level (e.g., ADI) or carcinogenic potential for single compounds based either on quantitative structure-activity relationships or on the results of short-term screening tests, such methods are not at present recommended as the sole basis of a risk assessment on chemical mixtures.

b. Exposure Uncertainties—The general uncertainties in exposure assessment have been addressed in the Agency's Guidelines for Estimating Exposures (U.S. EPA, 1986d). 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 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.

c. Uncertainties Regarding Composition of the Mixture—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, 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.

3. ASSUMPTIONS AND LIMITATIONS

3.1. INFORMATION ON INTERACTIONS

Most of the data available on toxicant interactions are derived from acute toxicity studies using experimental animals in which mixtures of two compounds were tested, often in only a single combination. Major areas of uncertainty with the use of such data involve the appropriateness of interaction data from an acute toxicity study for quantitatively altering a risk assessment for subchronic or chronic exposure, the appropriateness of interaction data on two component mixtures for quantitatively altering a risk assessment on a mixture of several compounds, and the accuracy of interaction data on experimental animals for quantitatively predicting interactions in humans.

The use of interaction data from acute toxicity studies to assess the potential interactions on chronic exposure is highly questionable unless the mechanisms of the interaction on acute exposure were known to apply to low-dose chronic exposure. Most known biological mechanisms for toxicant interactions, however, involve some form of competition between the chemicals or phenomena involving saturation of a receptor site or metabolic pathway. As the doses of the toxicants are decreased, it is likely that these mechanisms either no longer will exert a significant effect or will be decreased to an extent that cannot be measured or approximated.

The use of information from two-component mixtures to assess the interactions in a mixture containing more than two compounds also is questionable from a mechanistic perspective. For example, if two compounds are known to interact, either synergistically or antagonistically, because of the effects of one compound on the metabolism or excretion of the other, the addition of a third compound which either chemically alters or affects the absorption of one of the first two compounds could substantially alter the degree of the toxicologic interaction. Usually, detailed studies quantifying toxicant interactions are not available on multicomponent mixtures, and the few studies that are available on such mixtures (e.g., Gullino et al., 1956) do not provide sufficient information to assess the effects of interactive interference. Concerns with the use of interaction data on experimental mammals to assess interactions in humans is based on the increasing appreciation for systematic differences among species in their response to individual chemicals. If systematic differences in toxic sensitivity to single chemicals exist among species, then it seems reasonable to suggest that the magnitude of toxicant interactions among species also may vary in a systematic manner.

Consequently, even if excellent chronic data are available on the magnitude of toxicant interactions in a species of experimental mammal, there is uncertainty that the magnitude of the interaction will be the same in humans. Again, data are not available to properly assess the significance of this uncertainty.

Last, it should be emphasized that none of the models for toxicant interaction can predict the magnitude of toxicant interactions in the absence of extensive data. If sufficient data are available to estimate interaction coefficients as described in Section 4, then the magnitude of the toxicant interactions for various proportions of the same components can be predicted. The availability of an interaction ratio (observed response divided by predicted response) is useful only in assessing the magnitude of the toxicant interaction for the specific proportions of the mixture which was used to generate the interaction ratio.

The basic assumption in the recommended approach is that risk assessments on chemical mixtures are best conducted using toxicologic data on the mixture of concern or a reasonably similar mixture. While such risk assessments do not formally consider toxicologic interactions as part of a mathematical model, it is assumed that responses in experimental mammals or human populations noted after exposure to the chemical mixture can be used to conduct risk assessments on human populations. In bioassays of chemical mixtures using experimental mammals, the same limitations inherent in species-to-species extrapolation for single compounds apply to mixtures. When using health effects data on chemical mixtures from studies on exposed human populations, the limitations of epidemiologic studies in the risk assessment of single compounds also apply to mixtures. Additional limitations may be involved when using health effects data on chemical mixtures if the components in the mixture are not constant or if the components partition in the environment.

3.2. ADDITIVITY MODELS

If sufficient data are not available on the effects of the chemical mixture of concern or a reasonably similar mixture, the proposed approach is to assume additivity. Dose additivity is based on the assumption that the components in the mixture have the same mode of action and elicit the same effects. This assumption will not hold true in most cases, at least for mixtures of systemic toxicants. For systemic toxicants, however, most single compound risk assessments will result in the derivation of acceptable levels, which, as currently defined, cannot be adapted to the different forms of response additivity as described in Section 4.

Additivity models can be modified to incorporate quantitative data on toxicant interactions from subchronic or chronic studies using the models given in Section 4 or modifications of these models. If this approach is taken, however, it will be under the assumption that other components in the mixture do not interfere with the measured interaction. In practice, such subchronic or chronic interactions data seldom will be available. Consequently, most risk assessments (on mixtures) will be based on an assumption of additivity, as long as the components elicit similar effects.

Dose-additive and response-additive assumptions can lead to substantial errors in risk estimates if synergistic or antagonistic interactions occur. Although dose additivity has been shown to predict the acute toxicities of many mixtures of similar and dissimilar compounds (e.g., Pozzani et al., 1959; Smyth et al., 1969, 1970; Murphy, 1980), some marked exceptions have been noted. For example, Smyth et al. (1970) tested the interaction of 53 pairs of industrial chemicals based on acute lethality in rats. For most pairs of compounds, the ratio of the predicted LD_{50} to observed LD_{50} did not vary by more than a factor of 2. The greatest variation was seen with an equivolume mixture of morpholine and toluene, in which the observed LD_{50} was about five times less than the LD_{50} predicted by dose addition. In a study by Hammond et al. (1979), the relative risk of lung cancer attributable to smoking was 11, while the relative risk associated with asbestos exposure was 5. The relative risk of lung cancer from both smoking and asbestos exposure was 53, indicating a substantial synergistic effect. Consequently, in some cases, additivity assumptions may substantially underestimate risk. In other cases, risk may be overestimated. While this is certainly an unsatisfactory situation, the available data on mixtures are insufficient for estimating the magnitude of these errors. Based on current information, additivity assumptions are expected to yield generally neutral risk estimates (i.e., neither conservative nor lenient) and are plausible for component compounds that induce similar types of effects at the same sites of action.

4. MATHEMATICAL MODELS AND THE MEASUREMENT OF JOINT ACTION

The simplest mathematical models for joint action assume no interaction in any mathematical sense. They describe either dose addition or response addition and are motivated by data on acute lethal effects of mixtures of two compounds.

4.1. DOSE ADDITION

Dose addition assumes that the toxicants in a mixture behave as if they were dilutions or concentrations of each other, thus the true slopes of the dose-response curves for the individual compounds are identical, and the response elicited by the mixture can be predicted by summing the individual doses after adjusting for differences in potency; this is defined as the ratio of equitoxic doses. Probit transformation typically makes this ratio constant at all doses when parallel straight lines are obtained. Although this assumption can be applied to any model (e.g., the one-hit model in NRC, 1980b), it has been most often used in toxicology with the log-dose probit response model, which will be used to illustrate the assumption of dose addition. Suppose that two toxicants show the following log-dose probit response equations:

$$Y_1 = 0.3 + 3 \log Z_1 \qquad (4\text{-}1)$$
$$Y_2 = 1.2 + 3 \log Z_2 \qquad (4\text{-}2)$$

where Y_1 is the probit response associated with a dose of Z_1 (i = 1, 2). The potency, p, of toxicant #2 with respect to toxicant #1 is defined by the quantity Z_1/Z_2 when $Y_1 = Y_2$ (that is what is meant by equitoxic doses). In this example, the potency, p, is approximately 2. Dose addition assumes that the response, Y, to any mixture of these two toxicants can be predicted by

$$Y = 0.3 + 3 \log (Z_1 + pZ_2) \qquad (4\text{-}3)$$

Thus, since p is defined as Z_1/Z_2, Equation 4-3 essentially converts Z_2 into an equivalent dose of Z_1 by adjusting for the difference in potency. A more generalized form of this equation for any number of toxicants is:

$$Y = a_1 + b \log (f_1 + \Sigma f_i p_i) + b \log Z \qquad (4\text{-}4)$$

where:

a_1 = the y-intercept of the dose-response equation for toxicant #1
b = the slope of the dose-response lines for the toxicants
f_i = the proportion of the ith toxicant in the mixture
p_i = the potency of the ith toxicant with respect to toxicant #1 (i.e., Z_1/Z_i); and
Z = the sum of the individual doses in the mixture.

A more detailed discussion of the derivation of the equations for dose addition is presented by Finney (1971).

4.2. RESPONSE ADDITION

The other form of additivity is referred to as response addition. As detailed by Bliss (1939), this type of joint action assumes that the two toxicants act on different receptor systems and that the correlation of individual tolerances may range from completely negative (r = –1) to completely positive (r = + 1). Response addition assumes that the response to a given concentration of a mixture of toxicants is completely determined by the responses to the components and the pairwise correlation coefficient. Taking P as the proportion of organisms responding to a mixture of two toxicants which evoke individual responses of P_1 and P_2, then.

$$P = P_1 \text{ if } r = 1 \text{ and } P_1 \geq P_2 \qquad (4\text{-}5)$$
$$P = P_2 \text{ if } r = 1 \text{ and } P_1 < P_2 \qquad (4\text{-}6)$$
$$P = P_1 + P_2 (1 - P_1) \text{ if } r = 0 \qquad (4\text{-}7)$$
$$P = P_1 + P_2 \text{ if } r = -1 \text{ and } P \leq 1. \qquad (4\text{-}8)$$

More generalized mathematical models for this form of joint action have been given by Plackett and Hewlett (1948).

4.3. INTERACTIONS

All of the above models assume no interactions and therefore do not incorporate measurements of synergistic or antagonistic effects. For measuring toxicant interactions for mixtures of two compounds, Finney (1942) proposed the following modification of Equation 4-4 for dose addition:

$$Y = a_1 + b \log (f_1 + pf_2 + K [pf_1f_2]^{0.5}) + b \log Z \qquad (4\text{-}9)$$

where a_1, b, f_1, f_2, p, and Z are defined as before, and K is the coefficient of interaction. A positive value of K indicates synergism, a negative value indicates antagonism, and a value of zero corresponds to dose addition as in Equation 4-4. Like other proposed modifications of dose addition (Hewlett, 1969), the equation assumes a consistent interaction throughout the entire range of proportions of individual components. To account for such asymmetric patterns of interaction as those observed by Alstott et al. (1973), Durkin (1981) proposed the following modification to Equation 4-9:

$$Y = a_1 + b \log (f_1 + pf_2 + K_1f_1 [pf_1f_2]^{0.5} + K_2f_2[pf_1f_2]^{0.5}) + b \log z \qquad (4\text{-}10)$$

in which $K(pf_1f_2)^{0.5}$ is divided into two components, $K_1f_1 (pf_1f_2)^{0.5}$ and $K_2f_2[pf_1f_2]^{0.5}$. Since K_1 and K_2 need not have the same sign, apparent instances of antagonism at one receptor site and synergism at another receptor site can be estimated. When K_1 and K_2 are equal, Equation 4-10 reduces to Equation 4-9.

It should be noted that to obtain a reasonable number of degrees of freedom in the estimation of K in Equation 4-9 or K_1 and K_2 in Equation 4-10, the toxicity of several different combinations of the two components must be assayed along with assays of the toxicity of the individual components. Since this requires experiments with large numbers of animals, such analyses have been restricted for the most part to data from acute bioassays using insects (e.g., Finney, 1971) or aquatic organisms (Durkin, 1979). Also, because of the complexity of

experimental design and the need for large numbers of animals, neither Equation 4-9 nor Equation 4-10 has been generalized or applied to mixtures of more than two toxicants. Modifications of response-additive models to include interactive terms have also been proposed, along with appropriate statistical tests for the assumption of additivity (Korn and Liu, 1983; Wahrendorf et al., 1981).

In the epidemiologic literature, measurements of the extent of toxicant interactions, S, can be expressed as the ratio of observed relative risk to relative risk predicted by some form of additivity assumption. Analogous to the ratio of interaction in classical toxicology studies, $S = 1$ indicates no interaction, $S > 1$ indicates synergism, and $S < 1$ indicates antagonism. Several models for both additive and multiplicative risks have been proposed (e.g., Hogan et al., 1978; NRC, 1980b; Walter, 1976). For instance, Rothman (1976) has discussed the use of the following measurement of toxicant interaction based on the assumption of risk additivity:

$$S = (R_{11} - 1)/(R_{10} + R_{01} - 2) \qquad (4\text{-}11)$$

where R_{10} is the relative risk from compound #1 in the absence of compound #2, R_{01} is the relative risk from compound #2 in the absence of compound #1, and R_{11} is the relative risk from exposure to both compounds. A multiplicative risk model adapted from Walter and Holford (1978, Equation 4) can be stated as:

$$S = R_{11}/(R_{10}\, R_{01}) \qquad (4\text{-}12)$$

As discussed by both Walter and Holford (1978) and Rothman (1976), the risk-additive model is generally applied to agents causing diseases while the multiplicative model is more appropriate to agents that prevent disease. The relative merits of these and other indices have been the subject of considerable discussion in the epidemiologic literature (Hogan et al., 1978; Kupper and Hogan, 1978; Rothman, 1978; Rothman et al., 1980; Walter and Holford, 1978). There seems to be a consensus that for public health concerns regarding causative (toxic) agents, the additive model is more appropriate.

Both the additive and multiplicative models assume statistical independence in that the risk associated with exposure to both compounds in combination can be predicted by the risks associated with separate exposure to the individual compounds. As illustrated by Siemiatycki and Thomas (1981) for multistage carcinogenesis, the better fitting statistical model will depend not only upon actual biological interactions, but also upon the stages of the disease process which the compounds affect. Consequently, there is no a priori basis for selecting either type of model in a risk assessment. As discussed by Stara et al. (1983), the concepts of

multistage carcinogenesis and the effects of promoters and cocarcinogens on risk are extremely complex issues. Although risk models for promoters have been proposed (e.g., Bums et al., 1983), no single approach can be recommended at this time.

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PART B: RESPONSE TO PUBLIC AND SCIENCE ADVISORY BOARD COMMENTS

1. INTRODUCTION

This section summarizes some of the major issues raised in public comments on the Proposed Guidelines for the Health Risk Assessment of Chemical Mixtures published on January 9, 1985 (50 FR 1170). Comments were received from 14 individuals or organizations. An issue paper reflecting public and external review comments was presented to the Chemical Mixtures Guidelines Panel of the Science Advisory Board (SAB) on March 4, 1985. At its April 22-23, 1985, meeting, the SAB Panel provided the Agency with additional suggestions and recommendations concerning the Guidelines. This section also summarizes the issues raised by the SAB.

The SAB and public commentators expressed diverse opinions and addressed issues from a variety of perspectives. In response to comments, the Agency has modified or clarified many sections of the Guidelines, and is planning to develop a technical support document in line with the SAB recommendations. The discussion that follows highlights significant issues raised in the comments, and the Agency's response to them. Also, many minor recommendations, which do not warrant discussion here, were adopted by the Agency.

2. RECOMMENDED PROCEDURES

2.1. DEFINITIONS

Several comments were received concerning the lack of definitions for certain key items and the general understandability of certain sections. Definitions have been rewritten for several terms and the text has been significantly rewritten to clarify the Agency's intent and meaning.

Several commentators noted the lack of a precise definition of "mixture," even though several classes of mixtures are discussed. In the field of chemistry, the term "mixture" is usually differentiated from true solutions, with the former defined as nonhomogeneous multicomponent systems. For these Guidelines, the term "mixture" is defined as ". . any combination of two or more chemicals regardless of spatial or temporal homogeneity of source" (Section 1). These Guidelines are intended to cover risk assessments for any situation where the population is exposed or potentially exposed to two or more compounds of concern. Consequently, the introduction has been revised to clarify the intended breadth of application.

Several commentators expressed concern that "sufficient similarity" was difficult to define and that the Guidelines should give more details concerning similar mixtures. The Agency agrees and is planning research projects to improve on the definition. Characteristics such as

composition and toxic end-effects are certainly important, but the best indicators of similarity in terms of risk assessment have yet to be determined. The discussion in the Guidelines emphasizes case-by-case judgment until the necessary research can be performed. The Agency considered but rejected adding an example, because it is not likely that any single example would be adequate to illustrate the variety in the data and types of judgments that will be required in applying this concept. Inclusion of examples is being considered for the technical support document.

2.2. MIXTURES OF CARCINOGENS AND SYSTEMIC TOXICANTS

The applicability of the preferred approach for a mixture of carcinogens and systemic (noncarcinogenic) toxicants was a concern of several public commentators as well as the SAB. The Agency realizes that the preferred approach of using test data on the mixture itself may not be sufficiently protective in all cases. For example, take a simple two-component mixture of one carcinogen and one toxicant. The preferred approach would lead to using toxicity data on the mixture of the two compounds. However, it is possible to set the proportions of each component so that in a chronic bioassay 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 for the carcinogen to induce tumors in the small experimental group, the toxicant could induce mortality. At a lower dose in the same study, no adverse effects would be observed, including no carcinogenic effects. The data would then suggest use of a threshold approach. Since carcinogenicity is considered by the Agency to be a nonthreshold effect, it may not be prudent to construe the negative results of such a bioassay as indicating the absence of risk at lower doses. Consequently, the Agency has revised the discussion of the preferred approach to allow the risk assessor to evaluate the potential for masking of carcinogenicity or other effects on a case-by-case basis.

Another difficulty occurs with such a mixture when the risk assessment needs to be based on data for the mixture components. Carcinogens and systemic toxicants are evaluated by the Agency using different approaches and generally are described by different types of data: response rates for carcinogens vs. effect descriptions for toxicants. The Agency recognizes this difficulty and recommends research to develop a new assessment model for combining these dissimilar data sets into one risk estimate. One suggestion in the interim is to present separate risk estimates for the dissimilar end points, including carcinogenic, teratogenic, mutagenic, and systemic toxicant components.

3. ADDITIVITY ASSUMPTION

Numerous comments were received concerning the assumption of additivity, including:

a. the applicability of additivity to "complex" mixtures;
b. the use of dose additivity for compounds that induce different effects;
c. the interpretation of the Hazard Index; and
d. the use of interaction data.

Parts of the discussion in the proposed guidelines concerning the use of additivity assumptions were vague and have been revised in the final Guidelines to clarify the Agency's intent and position.

3.1. COMPLEX MIXTURES

The issue of the applicability of an assumption of additivity to complex mixtures containing tens or hundreds of components was raised in several of the public comments. The Agency and its reviewers agree that as the number of compounds in the mixture increases, an assumption of additivity will become less reliable in estimating risk. This is based on the fact that each component estimate of risk or an acceptable level is associated with some error and uncertainty. With current knowledge, the uncertainty will increase as the number of components increases. In any event, little experimental data are available to determine the general change in the error as the mixture contains more components. The Agency has decided that a limit to the number of components should not be set in these Guidelines. However, the Guidelines do explicitly state that as the number of compounds in the mixture increases, the uncertainty associated with the risk assessment is also likely to increase.

3.2. DOSE ADDITIVITY

Commentators were concerned about what appeared to be a recommendation of the use of dose additivity for compounds that induce different effects. The discussion following the dose additivity equation was clarified to indicate that the act of combining all compounds, even if they induce dissimilar effects, is a screening procedure and not the preferred procedure in developing a hazard index. The Guidelines were further clarified to state that dose (or response) additivity is theoretically sound, and therefore best applied for assessing mixtures of similar acting components that do not interact.

3.3. INTERPRETATION OF THE HAZARD INDEX

Several comments addressed the potential for misinterpretation of the hazard index, and some questioned its validity, suggesting that it mixes science and value judgments by using "acceptable" levels in the calculation. The Agency agrees with the possible confusion regarding its use and has revised the Guidelines for clarification. The hazard index is an easily derived restatement of dose additivity, and is, therefore, most accurate when used with mixture components that have similar toxic action. When used with components of unknown or dissimilar action, the hazard index is less accurate and should be interpreted only as a rough indication of concern. As with dose addition, the uncertainty associated with the hazard index increases as the number of components increases, so that it is less appropriate for evaluating the toxicity of complex mixtures.

3.4. USE OF INTERACTION DATA

A few commentators suggested that any interaction data should be used to quantitatively alter the risk assessment. The Agency disagrees. The current information on interactions is meager, with only a few studies comparing response to the mixture with that predicted by studies on components. Additional uncertainties include exposure variations due to changes in composition, mixture dose, and species differences in the extent of the interaction. The Agency is constructing an interaction data base in an attempt to answer some of these issues. Other comments concerned the use of different types of interaction data. The Guidelines restrict the use of interaction data to that obtained from whole animal bioassays of a duration appropriate to the risk assessment. Since such data are frequently lacking, at least for chronic or subchronic effects, the issue is whether to allow for the use of other information such as acute data, *in vitro* data, or structure-activity relationships to quantitatively alter the risk assessment, perhaps by use of a safety factor. The Agency believes that sufficient scientific upport does not exist for the use of such data in any but a qualitative discussion of possible synergistic or antagonistic effects.

4. UNCERTAINTIES AND THE SUFFICIENCY OF THE DATA BASE

In the last two paragraphs of Section II of the Guidelines, situations are discussed in which the risk assessor is presented with incomplete toxicity, monitoring, or exposure data. The SAB, as well as several public commentors, recommended that the "risk management" tone of this section be modified and that the option of the risk assessor to decline to conduct a risk assessment be made more explicit.

This is a difficult issue that must consider not only the quality of the available data for risk assessment, but also the needs of the Agency in risk management. Given the types of poor

data often available, the risk assessor may indicate that the risk assessment is based on limited information and thus contains no quantification of risk. Nonetheless, in any risk assessment, substantial uncertainties exist. It is the obligation of the risk assessor to provide an assessment, but also to ensure that all the assumptions and uncertainties are articulated clearly and quantified whenever possible.

The SAB articulated several other recommendations related to uncertainties, all of which have been followed in the revision of the Guidelines. One recommendation was that the summary procedure table also be presented as a flow chart so that all options are clearly displayed. The SAB further recommended the development of a system to express the level of confidence in the various steps of the risk assessment.

The Agency has revised the summary table to present four major options: risk assessment using data on the mixture itself, data on a similar mixture, data on the mixture's components, or declining to quantify the risk when the data are inadequate. A flow chart of this table has also been added to more clearly depict the various options and to suggest the combining of the several options to indicate the variability and uncertainties in the risk assessment.

To determine the adequacy of the data, the SAB also recommended the development of a system to express the level of confidence associated with various steps in the risk assessment process. The Agency has developed a rating scheme to describe data quality in three areas: interaction, health effects, and exposure. This classification provides a range of five levels of data quality for each of the three areas. Choosing the last level in any area results in declining to perform a quantitative risk assessment due to inadequate data. These last levels are described as follows:

Interactions: An assumption of additivity cannot be justified, and no quantitative risk assessment can be conducted.

Health effects: A lack of health effects information on the mixture and its components precludes a quantitative risk assessment.

Exposure: The available exposure information is insufficient for conducting a risk assessment.

Several commentors, including the SAB, emphasized the importance of not losing these classifications and uncertainties farther along in the risk management process. The discussion of uncertainties has been expanded in the final Guidelines and includes the recommendation that a

discussion of uncertainties and assumptions be included at every step of the regulatory process that uses risk assessment.

Another SAB comment was that the Guidelines should include additional procedures for mixtures with more than one end point or effect. The Agency agrees that these are concerns and revised the Guidelines to emphasize these as additional uncertainties worthy of further research.

5. NEED FOR A TECHNICAL SUPPORT DOCUMENT

The third major SAB comment concerned the necessity for a separate technical support document for these Guidelines. The SAB pointed out that the scientific and technical background from which these Guidelines must draw their validity is so broad and varied that it cannot reasonably be synthesized within the framework of a brief set of guidelines. The Agency is developing a technical support document that will summarize the available information on health effects from chemical mixtures, and on interaction mechanisms, as well as identify and develop mathematical models and statistical techniques to support these Guidelines. This document will also identify critical gaps and research needs.

Several comments addressed the need for examples on the use of the Guidelines. The Agency has decided to include examples in the technical support document.

Another issue raised by the SAB concerned the identification of research needs. Because little emphasis has been placed on the toxicology of mixtures until recently, the information on mixtures is limited. The SAB pointed out that identifying research needs is critical to the risk assessment process, and the EPA should ensure that these needs are considered in the research planning process. The Agency will include a section in the technical support document that identifies research needs regarding both methodology and data.

APPENDIX B
DEFINITIONS

Consistent and clear terminology is critical to the discussion of chemical mixtures risk assessment methodology. Tables A-1 and A-2 articulate the differences among the many terms used to describe chemical mixtures and the types of interactions that may occur among chemicals. Table A-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, environmental source of the mixture, toxic endpoint, etc. Table A-2 provides definitions for terms that describe various types of toxicologic interactions, including forms of additivity, antagonism, synergism, and other toxicologic phenomena. Tables A-1 and A-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.

Table B-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 coexist 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 similarly shaped dose-response curves, or parallel log dose-probit response curves for quantal data on the number of animals (people) responding, 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 B-2. Definitions of toxicologic interactions between chemicals[a]
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 each chemical behaves as a concentration or dilution of every other chemical in the mixture. The response of the combination is the response expected from the equivalent dose of an index chemical. The equivalent dose is the sum of component doses scaled by their toxic 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 B-2. Definitions of toxicologic interactions between chemicals[a] (continued)
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 toxic 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. For two chemical mixtures, the body's response to the first chemical is the same whether or not the second chemical is present.
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.

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

APPENDIX C
PHARMACOKINETICS

The improvement in predictive estimates for mixture risk will likely follow the increase in biological understanding and quantitative models of toxicologic interactions. The best studied and modeled toxicologic interactions are those involving alterations in pharmacokinetics (e.g., see Krishnan et al., 1994). This section discusses the general concepts underlying toxicologic interactions and more specific issues with pharmacokinetic models to provide background and incentive for continued research in this area.

C.1. PHARMACOKINETIC/PHARMACODYNAMIC MODELING

The past 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 might 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 modes of action or 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: those resulting from toxicokinetic and those resulting from toxicodynamic modes of action. Toxicokinetic modes 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 modes of interaction include interactions that do not directly affect the metabolism or disposition of a xenobiotic, but affect a tissue's response or susceptibility to toxic injury. Modes of toxicodynamic interactions include, among others, depletion or induction of protective factors, alterations in tissue repair, changes in hemodynamics, and immunomodulation. Sauer and Sipes (1995) have reported toxicodynamic action between all-*trans*-retinol and other chemicals that 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 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 (PBPK) modeling in predicting the consequences of exposure to multiple solvents has been demonstrated by Krishnan and Pelekis

(1995). The authors used PBPK models and existing data sets 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 modes of action of toxicity in order to effectively use PBPK 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 the 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 (PBPK/PD) may therefore be considered a viable approach. Tardif et al. (1997) developed a PBPK 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 PBPK models in the prediction of the kinetics of components of chemical mixtures, by accounting for modes of interaction of binary chemical mixtures.

The linkage of two of the most challenging areas in toxicology today, PBPK/PD and statistical/mathematical modeling and experimental toxicology of chemical mixtures, will have immense potential in application to risk assessment for chemical mixtures. Figure B-1 represents the possible application of combined PBPK/PD modeling to chemical mixtures and the development of innovative risk assessment methodologies for chemical mixtures. El-Masri et al. (1996) attempted to couple PBPK/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

A Priori PBPK/PD Modeling

↓

Model-Directed Focused Experiments/
Efficient Experimental Designs

↓

PBPK/PD Integrated Toxicity Model	+	Isobolographic Analysis Response Surface Methodology Monte Carlo Simulation

↓

Predictive and Alternative Toxicology/
Target Tissue Dosimetry

↓

Innovative Risk Assessment
Methodologies

Figure C-1.
The possible application of combined PBPK/PD modeling to chemical mixtures.

as benchmark doses, human risk assessment of chemical mixtures may possibly be carried out with quantification of the uncertainty.

C.2. PHARMACOKINETIC PRINCIPLES: CHEMICAL MIXTURES

Environmental exposures to naturally occurring and artificially produced substances generally involve 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, may not move from the tissue or even the intracellular site where they were produced. Given this broad spectrum of modes 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 modes of kinetic interaction during absorption and elimination; the following discussions summarize this review and include other pertinent information available in the current literature.

C.2.1. Absorption

C.2.1.1. *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 a 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 a 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 the 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 processes. 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 at properly spaced time intervals (Neuvonen, 1976).

C.2.1.2. *Pulmonary*

Gaseous and particulate phases of an inhaled chemical mixture may play different functional roles inducing or reducing pulmonary/systemic toxicity. For example, formaldehyde can stimulate mucociliary function at low concentrations, but it inhibits mucociliary 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, chronic exposures lower 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, such as 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. Because 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.

C.2.1.3. *Dermal*

Despite lack of sufficient quantitative information, solvent effects on qualitative absorption for the 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 that 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, which have been deposited on yards and playgrounds. Adults and children can also be exposed to 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 the contaminated chemical on laboratory animals or use 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 that 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 deserves 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.

C.2.1.4. *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) systems, 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).

Andersen et al. (1987), while developing a PBPK model, considered the interaction between 1,1-dichloroethylene (1,1-DCE) and trichloroethylene (TCE) 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 led 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 enzymes may produce different effects on metabolic rates, which could reduce integrated exposure to the parent chemical by increasing the 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 modes of action 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 more than 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 sbiphasic interactive effects that must be considered in predictive studies.

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