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Developing Relative Potency Factors for Pesticide Mixtures: Biostatistical Analyses of Joint Dose-Response

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NOTICE

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to the Agency's peer and administrative review and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

This report was developed by the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD), National Center for Environmental Assessment - Cincinnati Office (NCEA-Cin) in collaboration with EPA's Office of Pesticide Programs. It contains information concerning biological concepts and statistical procedures for improving the application of Relative Potency Factors (RPFs) to pesticide mixtures. This research supports the need for chemical mixtures risk assessment research as mandated in 1996 by both the Food Quality Protection Act (FQPA) and the Safe Drinking Water Act Amendments. Research results are presented regarding the theoretical basis for RPF-based risk assessments; new quantitative methods for applying RPFs are shown. The RPF approach assumes toxicity of the mixture components can be characterized using dose addition. Thus, the basic tenets of dose addition, common toxic modes of action and similarly-shaped dose-response curves among the mixture components, are investigated and discussed. This research was undertaken to continue exploring and developing cumulative risk assessment strategies beyond current applications and is intended to improve future applications of RPF based risk assessments.

The statistical methods presented in this effort are based on research conducted by Jim Chen, Yi-Ju Chen, and Ralph Kodell through an Interagency Agreement between EPA and the Food and Drug Administration's National Center for Toxicological Research. An external review was conducted by Drs. Christine F. Chaisson, Pavel Muller, and Walter W. Peigorsch under EPA Contract No. 68-C-02-060/061 with Versar, Inc.

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LIST OF ABBREVIATIONS

CRA	Cumulative Risk Assessment
DBPs	Disinfection By-Products
ED	Effective Dose
EPA	Environmental Protection Agency
FQPA	Food Quality Protection Act
HI	Hazard Index
ICED	Index Chemical Equivalent Dose
LOAEL	Lowest-Observed-Adverse-Effect Level
MF	Modifying Factor
NOAEL	No-Observed-Adverse-Effect Level
OP	Organophosphorus Pesticide
ORD	Office of Research and Development
PBPK	Physiologically-Based Pharmacokinetic
RAF	Risk Assessment Forum
RfD	Reference Dose
RPF	Relative Potency Factor
TEF	Toxicity Equivalence Factor
TEQ	2,3,7,8-TCDD Toxicity Equivalents
UF	Uncertainty Factor

KEY DEFINITIONS

Absorbed Dose - the amount of a substance crossing a specific barrier through uptake processes.[1]

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").[3]

Bioavailability - The state of being capable of being absorbed and available to interact with the metabolic processes of an organism. Bioavailability is typically a function of chemical properties, physical state of the material to which an organism is exposed, and the ability of the individual organism to physiologically take up the chemical.[1]

Chemical Classes - Groups of components that exhibit similar biologic activities, 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.[3] (Note: this is slightly modified from the original version).

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.”[3]

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.[3]

Components - Single chemicals that make up a chemical mixture that may be further classified as systemic toxicants, carcinogens, or both.[3]

Dose Additivity - When the effect of the combination is the effect expected from the equivalent dose of an index chemical. The equivalent dose is the sum of component doses scaled by their potency relative to the index chemical.[3]

Dose - The amount of a substance available for interaction with metabolic processes or biologically significant receptors after crossing the outer boundary of an organism[1].

Dose-Response Assessment - A determination of the relationship between the magnitude of an administered, applied, or internal dose and a specific biological response. Response can be expressed as measured or observed incidence, percent response in groups of subjects (or populations), or as the probability of occurrence within a population.[2]

Dose-Response Relationship - The relationship between a quantified exposure (dose), and the proportion of subjects demonstrating specific, biological changes (response).[2] U.S. EPA's draft 1996 Cancer Guidelines further state: "Whether animal experiments or epidemiologic studies are the sources of data, questions need to be addressed in arriving at an appropriate measure of dose for the anticipated environmental exposure. Among these are:

- whether the dose is expressed as an environmental concentration, applied dose, or delivered dose to the target organ,
- whether the dose is expressed in terms of a parent compound, one or more metabolites, or both,
- the impact of dose patterns and timing where significant,
- conversion from animal to human doses, where animal data are used, and
- the conversion metric between routes of exposure where necessary and appropriate."

Effective Dose (ED_{10}) - The dose corresponding to a 10% increase in an adverse effect, relative to the control response.[2]

Exposure - Contact made between a chemical, physical, or biological agent and the outer boundary of an organism. Exposure is quantified as the amount of an agent available at the exchange boundaries of the organism (e.g., skin, lungs, gut).[2]

Exposure Assessment - An identification and evaluation of the human population exposed to a toxic agent, describing its composition and size, as well as the type, magnitude, frequency, route and duration of exposure.[2]

Extrapolation, low dose - An estimate of the response at a point below the range of the experimental data, generally through the use of a mathematical model.[2]

Human Equivalent Concentration (HEC) or Dose (HED) - The human concentration (for inhalation exposure) or dose (for other routes of exposure) of an agent that is believed to induce the same magnitude of toxic effect as the experimental animal species concentration or dose. This adjustment may incorporate toxicokinetic information on the particular agent, if available, or use a default procedure, such as assuming that daily oral doses experienced for a lifetime are proportional to body weight raised to the 0.75 power.[2]

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.[3]

Index Chemical Equivalent Dose -The exposure to a chemical that is expected to elicit the same response as that of the index chemical, when the chemicals are administered by the same route, at the same duration and frequency. The chemical and the index chemical must share a common mode of action.

Internal dose - A more general term denoting the amount absorbed without regard to absorption process.[1]

Independence of Action - 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.[3]

Mechanism of Toxicity or Mechanism of Toxic Action - The set of molecular and cellular events leading to a toxicologic outcome. [A toxicologic outcome is considered to be damage to the organism at any level of biological organization (i.e., molecular, cellular, tissue,...).][4]

Mode of Action - The set of biological events at the target tissue or target organ leading to a toxicologic outcome. [A toxicologic outcome is considered to be damage to the organism at any level of biological organization (i.e., molecular, cellular, tissue,...).][4]

Model - A mathematical function with parameters that can be adjusted so the function closely describes a set of empirical data. A mechanistic model usually reflects observed or hypothesized biological or physical mechanisms, and has model parameters with real world interpretation. In contrast, statistical or empirical models selected for particular numerical properties are fitted to data; model parameters may or may not have real world interpretation. When data quality is otherwise equivalent, extrapolation from mechanistic models (e.g., biologically based dose-response models) often carries higher confidence than extrapolation using empirical models (e.g., logistic model).[2]

Physiologically Based Pharmacokinetic (PBPK) Model - Physiologically based compartmental model used to characterize pharmacokinetic behavior of a chemical. Available data on blood flow rates, and metabolic and other processes which the chemical undergoes within each compartment are used to construct a mass-balance framework for the PBPK model.[2]

Point of Departure - The dose-response point that marks the beginning of a low-dose extrapolation. This point is most often the upper bound on an observed incidence or on an estimated incidence from a dose-response model.[2]

Risk - The probability of deleterious effects on health.[1]

Relative Potency Factor Method - A mixtures risk assessment approach used to assess risks posed by mixture components that exhibit a common mode of action. The toxic potency of each mixture component is compared to that of an index chemical generating a measure of potency for each component that is *relative* to the toxicity of the index chemical. For application, the shapes of the individual component dose-response functions must be similarly over the region of the mixture exposure.

Response Additivity - When the response (rate, incidence, risk, or probability) of effects from the combination is equal to the conditional sum of component responses as defined by the formula for the sum of independent event probabilities.[3]

Similar Components - Single chemicals that cause the same biologic activity or are expected to cause a type of biologic activity based on chemical structure. Evidence of similarity may include parallel log-probit dose-response curves and same mechanism of action or toxic endpoint. These components are expected to have comparable characteristics for fate, transport, physiologic processes, and toxicity.[3]

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.[3]

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.[3]

Target Organ - The biological organ(s) most adversely effected by exposure to a chemical substance.[2]

Uptake - The process by which a substance crosses an absorption barrier and is absorbed into the body.[1]

Sources

[1]U.S. EPA. 1992. Guidelines for Exposure Assessment; Notice. Federal Register. 57(104):22888-22938.

[2]U.S. EPA. 2003. Integrated Risk Information System. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Online. http://www.epa.gov/iris

[3]U.S. EPA. 2001. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. Office of Research and Development, Washington, DC. EPA/630/R-00/002. Available in PDF format at: www.epa.gov/NCEA/raf/chem_mix.htm

[4]U.S. EPA. 2002. The Feasibility of Performing Cumulative Risk Assessments for Mixtures of Disinfection By-Products in Drinking Water. NCEA-C-1257. Final Draft.

EXECUTIVE SUMMARY

Cumulative Risk Assessment (CRA) is defined in U.S. EPA's Risk Assessment Forum (RAF) CRA Framework (U.S. EPA, 2002a) as "the combined risks from aggregate exposures to multiple agents or stressors." CRA has become an important research area, reflecting the interest of U.S. EPA's regional risk assessors, program offices, Office of Environmental Justice, and Office of Children's Risk. In implementing the requirements of the Food Quality Protection Act of 1996, U.S. EPA's Office of Pesticide Programs has developed guidance for conducting CRA's of chemicals that appear to act by a common mechanism of toxicity (U.S. EPA, 2002b). Because the organophosphorus pesticides (OPs) are considered to exert some of their toxic effects via a common toxicologic mechanism (i.e., cholinesterase inhibition), these compounds have been the subject of a CRA (U.S. EPA, 2001b). Additional CRA's may be performed on additional pesticide classes (e.g., triazinines, carbamates) and other co-occurring substances for which a common mode of action can be identified. The risk assessment method employed in the OP cumulative risk study is the Relative Potency Factor (RPF) approach (U.S. EPA, 2000). Dose addition is the critical methodological assumption, requiring the mixture components to act by the same toxic mode of action and to have similarly-shaped dose-response curves.

Assessing the cumulative toxicological effects of multiple chemicals has been addressed from time to time (NRC, 1988; U.S. EPA, 1986, 2000). Methods and data that can be used to estimate the risk of exposures to multiple chemicals have been developed. Although U.S. EPA guidance exists regarding the basic theory for RPFs,

the toxicological criteria for defining and determining a common mode of action among chemicals continue to need refinement; results on this subject are presented in this report. Further, results are presented on appropriate statistical methods for CRA, based on research published in Chen et al. (2001, 2003). Biostatistical approaches are shown for grouping chemicals identified as having common modes of action, proposing two classification algorithms to cluster chemicals into subclasses within which chemicals have similarly-shaped dose-response functions. Chemicals within subclasses are combined using the RPF method when a constant relative potency among chemicals exists. Additional methods are shown to calculate cumulative risks inclusive of these subclasses (i.e., combining across subclasses for which a non-constant relative potency exists) using either a joint dose-response approach or by integrating the concepts of dose addition and response addition.

An important question in mixtures risk assessment research is how to assess a mixture containing some chemicals that share a common toxic mode of action and other chemicals that do not. Current additivity methods have evolved to handle either the former (dose addition) or the latter (response addition). Alternatively, the risk assessor may choose to do the assessment based on whole mixture data. The biostatistical methods developed in this report provide alternative methods to evaluate a mixture under three scenarios. The simple case occurs when there is certainty that a common toxic mode of action is operating, so a dose addition approach can be applied. The second case occurs when the mixtures can be divided into independent mode of action subclasses; dose addition and response addition can be integrated to make the assessment. The third case occurs when mode of action is uncertain, so a joint

dose-response modeling procedure is used to create a range of risk estimates. Thus, these approaches enrich the available library of mixture risk assessment methods beyond what is currently published by the U.S. EPA (1986, 2000). Further, these approaches may be useful in future assessments of pesticide mixtures to be evaluated under FQPA. Finally, the results presented here are generalizable to assessments of other environmental mixtures; the risk assessments that support environmental regulations of important environmental mixtures such as dioxins, polychlorinated biphenyls, and OPs are based on concepts of additivity (U.S. EPA, 1989b, 2000, 2001b).

The research results in this report can be applied to reduce uncertainties in RPF-based risk assessments of chemical mixtures. These results also show how mixtures risk assessments can be conducted using additivity concepts. Various sources of uncertainty exist in most mixtures risk assessments, including uncertainties addressed in this report regarding several factors:

- Common mode of action across mixture components (Sections 2, 3)
- Similarly shaped dose-response curves across mixture components (Sections 2, 5)
- Value of internal vs. external dose estimates for developing RPFs (Section 4)
- Choice of dose metric (moles vs. mass) to use in a cumulative risk assessment (Section 4)
- Cross-species extrapolation of relative potency factors (Section 4)
- Estimating risks for a mixture with two or more common mode of action subclasses (Section 5).

Biostatistical modeling in this report presents ways to combine dose-response information, partitioning the mixtures into common mode of action subclasses. These models can then be used to estimate risks for specific exposure scenarios or used to develop toxicity values, such as a reference dose for the mixture. Three RPF-based methods are discussed, reflecting what is known or uncertain about the mixture toxicology. These approaches can be applied using internal or external doses. Development of approaches based on internal doses may reduce some toxicokinetic uncertainties associated with RPFs based on administered doses. In the Chen et al. papers (2001, 2003) in Appendices A and B, external doses were used to develop statistical methods for grouping components into common mode of action subclasses. The next step in this process is to use RPFs based on internal doses and compare subclass groupings and modeling results with those developed using external doses. Recommendations for future RPF research on pesticide mixtures are listed here.

1) Develop kinetic models for pesticide mixtures in rodents.

2) Using experimental cholinesterase inhibition measures, determine RPFs based on both external and internal dose estimates for the rodent.

3) Determine if the RPFs based on internal dose estimates significantly differ from RPFs developed from external doses for the rodent.

4) Apply the biostatistical methods for grouping by common dose-response curves using RPFs based on internal and external doses and compare the groupings that result.

5) Develop kinetic models for pesticide mixtures in humans.

6) Estimate human risks using rodent cholinesterase inhibition responses, RPFs based on rodent internal doses, and human internal dose estimates using the three approaches presented in Chen et al. (2001, 2003), as appropriate.

7) Compare subclass groupings and human risk estimates for all scenarios of internal and external RPFs.

8) Ealuate the toxicity of different human exposure scenarios with the RPF models developed.

This research was undertaken to continue exploring and developing cumulative risk assessment strategies based on dose addition concepts beyond current applications and is intended to improve future applications of RPF based risk assessments.

1. INTRODUCTION

The U.S. Environmental Protection Agency (U.S. EPA) and other regulatory agencies use risk assessment to evaluate the risk posed to humans through chemical exposures to contaminants in food, drinking water, or environmental media. Risk assessment for toxic agents is often conducted to evaluate the potential risks from exposure to a single toxic agent through a single route of exposure. Although it is important to evaluate individual toxic agents, people frequently are exposed to many chemicals simultaneously or in sequence by different exposure routes. These exposures to multiple chemicals through various media could cause unexpected cumulative effects. The combined risk from such exposures may be greater or less than what would typically be predicted from data on individual chemicals. Assessing the cumulative toxicological effects of multiple chemicals has been addressed from time to time (NRC, 1988; U.S. EPA, 1986, 2000). However, new methods and improvements to existing approaches are still needed to estimate risk from exposures to multiple chemicals.

Cumulative Risk Assessment (CRA) is defined in U.S. EPA's Risk Assessment Forum (RAF) CRA Framework (U.S. EPA, 2002a) as "the combined risks from aggregate exposures to multiple agents or stressors." CRA can include both chemical and non-chemical stressors, multiple-route exposures, population factors that differentially affect exposure or toxicity, and community based assessments. CRA has become an important research area, reflecting the interest of U.S. EPA's regional risk assessors, program offices, Office of Environmental Justice, and Office of Children's

Risk. In 2002, U.S. EPA's Office of Research and Development (ORD) jointly sponsored a workshop with U.S. EPA's Regions to discuss current case studies, methods and research needs regarding CRA (U.S. EPA, 2003a). Regional scientists are confronted with conducting community-based CRA's (e.g., assessing risks from multi-media, multi-stressor exposures to a population in a specified geographic area). Successful completion of such assessments require development of new data, methods, and guidance.

U.S. EPA's Program Offices generally conduct CRA's on a select group of co-occurring chemicals, and set broad national standards. Examples of programmatic interests include:

- The Office of Water needs to conduct chemical mixtures research to support requirements of the Safe Drinking Water Act Amendments of 1996 (U.S. EPA, 1996).
- The Office of Air Quality Planning and Standards has used a CRA approach in conducting the National Air Toxics Assessment of 33 air pollutants (a subset of 32 air toxics from the Clean Air Act's list of 188 air toxics plus diesel particulate matter) (U.S. EPA, 2001a).
- The Office of Solid Waste and Emergency Response assesses contaminant mixtures at Superfund Sites (U.S. EPA, 1989a) under the Comprehensive Environmental Response, Compensation, and Liability Act (U.S. EPA, 1980).
- The Office of Pesticide Programs has conducted a CRA on organophosphorus pesticide (OP) mixtures (U.S. EPA, 2001b), under the Food Quality Protection Act (FQPA) of 1996 (U.S. EPA, 1997). Case studies may be performed on additional pesticide classes (e.g., triazinines, carbamates) and other co-occurring substances for which a common mode of action can be identified.

The FQPA is the most specific act regarding CRA, requiring EPA to consider the potential human health risks of multiple route exposures to multiple pesticide residues

and substances that have a common mechanism of toxicity (U.S. EPA, 1997).[1] The first pesticide group to be evaluated (U.S. EPA, 2002b) is the organophosphorus pesticides (OPs), a group of closely related pesticides that affect nervous system function. They are applied to many food crops, as well as to residential and commercial buildings and lawns. The many uses of this class of pesticides result in frequent and consistent human exposures. The acute and chronic effects of OPs in humans, wild animals, and test animals are well known. OPs are neurotoxic because they bind to and phosphorylate the enzyme acetylcholinesterase in both the central (brain) and peripheral nervous systems, reducing the ability of the enzyme cholinesterase to function properly in regulating acetylcholine, a neurotransmitter. Acetylcholine is a critical factor in the transfer of nerve impulses from a nerve cell to a muscle cell or another nerve cell. If acetylcholine levels are not properly reduced by cholinesterase, the nerve impulses or neurons remain active longer than they should, overstimulating the nerves and muscles and causing toxic effects at many sites, including neuromuscular junctions and synapses of the central and autonomic nervous system.

As part of the implementation of FQPA, U.S. EPA's Office of Pesticide Programs has developed guidance for conducting cumulative risk assessments of chemicals that appear to act by a common mechanism of toxicity (U.S. EPA, 2002b). Because the OPs are considered to exert some of their toxic effects via a common toxicologic

[1]The terms *mechanism of toxicity* (or *mechanism of toxic action*) and *mode* of *action* represent a continuum of understanding regarding a toxicodynamic process (U.S. EPA, 2002c). A toxicologic outcome is considered to be damage to the organism at any level of biological organization (i.e., molecular, cellular, tissue,...). Knowledge of a chemical's *mechanism of toxicity or mechanism of toxic action* implies that the molecular and cellular events leading to a toxicologic outcome are described and well-understood. Knowledge of a chemical's *mode of action* implies a general understanding of the key toxicodynamic events that occur at a tissue level, but not a detailed description of these events at the cellular or molecular level. Mode of action is defined as the set of biological events at the target tissue or target organ leading to a toxicologic outcome.

mechanism (i.e. cholinesterase inhibition), these compounds have been the subject of a CRA (U.S. EPA, 2001b). The risk assessment method employed in the OP CRA and likely to be used in future pesticide CRA's is the Relative Potency Factor (RPF) approach (U.S. EPA, 2000). This report examines the theoretical basis for the RPF method, providing useful information to improve and enhance such future applications.

The RPF approach is appropriate under FQPA because dose addition is the critical RPF methodological assumption; implementation requires that the mixture components act by the same toxic mode of action. As explained in Section 2, a theoretical consequence of this assumption is that the components should have similarly-shaped dose-response curves between the response threshold and the maxima. To summarize the procedure, doses of mixture components are scaled by their potency relative to a well-studied component of the chemical mixture (referred to as the index chemical) using scaling factors called RPFs. The product of each mixture component's dose and its RPF is considered to be its equivalent dose in units of the index chemical. These dose equivalents of all the mixture components are summed to express the total mixture dose in terms of an Index Chemical Equivalent Dose (ICED).[2] The risk posed by the mixture is then quantified by comparing the mixture's ICED to the dose-response assessment of the index chemical. To implement this approach, the index chemical must have an adequate toxicologic dose-response data set.

U.S. EPA (2000) characterized the RPF methodology as a generalized form of the toxicity equivalence factor (TEF) methodology that has been used to assess risks

[2]The ICED has the same mathematical interpretation as the dioxin toxicity equivalents (TEQ). TEQ refers to the quantification of dioxin concentrations based on the congeners' equivalent 2,3,7,8-TCDD toxicity (U.S. EPA, 1989b). ICED is applied to mixtures other than dioxins.

posed by some dioxins (U.S. EPA, 1989b). The TEF approach uses a single TEF for each dioxin congener, applying this same TEF to all exposure routes, health effects, and exposure durations. The RPF methodology was developed for application to a broad set of chemical groups whose data sets are either less complete than the dioxins or indicate more variation in mode of action across route, effects and duration. The significant generalizations in the RPF methodology include the following:

1. ***RPFs may be developed to assess risks for a subset of the health effects caused by a mixture's components.*** For example, the same mixture components may be shown to cause both hepatotoxicity and renal toxicity in bioassays. Different RPFs may be developed to address the risk of each type of toxicity following human exposures. Mixture Component A may exhibit greater hepatotoxicity than Component B when compared to Index Chemical C; to reflect this, the RPF for the hepatotoxicity of Component A should be greater than the RPF of Component B. However, mixture Component B may exhibit greater renal toxicity than Component A when compared to Index Chemical C and, to reflect this, the RPF for the renal toxicity of Component A should be less than that of Component B.

   Note that some mixture components may act through multiple modes of action on different target tissues. It is conceivable that several RPFs may need to be developed to adequately address the risks posed by human exposures to the mixture. Thus, the membership of component chemicals may differ across groups of RPFs and may also overlap.

2. ***RPFs may be developed to assess risk for a single route of exposure.*** For example, the same mixture components may pose risk through inhalation and oral exposures. Based on differences in the *relative* toxicity of the components measured in inhalation and oral bioassays, different RPFs may be developed to address the human health risks following inhalation or oral exposures.

3. ***RPFs may be developed to assess risks for different durations of exposure.*** The toxicity of a group of mixture components may change *relative* to each other depending on the duration and frequency of the exposures. Different RPFs may be developed to address the human health risks following different exposure frequencies or exposure durations (e.g., different RPFs may be developed for exposures that achieve steady-state tissue concentrations of mixture components than for those exposures that do not result in steady-state tissue concentrations of the mixture components over the duration of the experiment).

4. ***RPFs may be developed to assess risks within a restricted range of dose levels of the mixture's components.*** The toxicities of different chemicals relative to each other may change with dose. For example, at higher dose levels where significant adverse responses are observed, an assumption of additivity may not be appropriate (i.e., observed effects may be greater than or less than those expected under an assumption of additivity). Thus, it is appropriate to restrict the dose range of the components in two ways: limit the range to levels for which additivity is an appropriate assumption and, ensure the range reflects the exposure levels of interest to the risk assessment. Different RPFs may be developed to assess risks to humans for these different ranges.

These generalizations of the TEF methodology allow RPF development to be limited to specific aspects of mixture toxicity and exposure, allowing the RPF approach to be more broadly applied.

An identified research need for the RPF methodology is continued development of appropriate statistical methods to support the assumption of a common toxic mode of action. One way to examine this assumption is to evaluate the similarity of the dose response curves across the mixture's components. Components with similar dose response curves can be grouped together into a mode of action subclass for which an RPF-based risk assessment can be developed.

Chen et al. (2001, 2003) present biostatistical approaches for grouping chemicals suspected to have common modes of action, proposing two classification algorithms to cluster chemicals into subclasses within which chemicals have similarly-shaped dose-response functions. Chemicals within subclasses are combined using the RPF method when a constant relative potency among chemicals exists. Additional methods are shown to calculate cumulative risks inclusive of these subclasses (i.e., combining across subclasses for which a non-constant relative potency exists) using either a joint dose-response approach or by integrating the concepts of dose addition and response addition.

Users of the RPF approach should appreciate that this model of mixtures toxicity is actually a fairly simplistic depiction of the risk posed by the mixture. Theoretically, the number of mixture components that can be included in an RPF-based approach is unlimited, as long as each component is truly a toxicologic clone of the index chemical. Pragmatically, there are a number of limitations including the availability of relevant

toxicologic data upon which to base the RPFs. The Chen et al. biostatistical methods were developed for pesticide mixtures. Pesticide mixtures are unusual among environmental mixtures because component toxicologic data are often available due to the laws that govern U.S. pesticides. These approaches can likely be used on as many as 30 or so individual components. The key limitations are having data describing the dose-response function for each component and toxicologic evidence that each component shares a common toxic mode-of-action. A statistical issue is caused by the toxic potency weighting of the exposure levels. If a poorly studied (high uncertainty) chemical has high potency, its equivalent dose is high with no discounting for the uncertainty. As the number of components increases, there may be an increased likelihood of such a dominant uncertainty. This emphasizes the need for careful discussion of uncertainties: their sources and impact on the final risk assessment.

This report presents research results regarding the theoretical basis for RPF based risk assessments and presents quantitative methods for applying RPFs. The two basic assumptions of dose addition, common toxic modes of action and similarly-shaped dose-response curves, are investigated and discussed. Research results produced by Chen et al. are presented, showing the integration of this research with applications of the RPF approach. This research was undertaken to continue exploring and developing cumulative risk assessment strategies beyond current applications and is intended to improve future applications of RPF based risk assessments.

2. DOSE ADDITION CONCEPTS

U.S. EPA guidance documents on chemical mixtures risk assessment (U.S. EPA, 1986, 2000) recommend no-interaction approaches under dose addition for the risk assessment of mixtures of toxicologically similar chemicals. Assuming the chemicals in a mixture are noninteractive and elicit a common response through similar actions on a biological system, the chemicals are then assumed to act as if one is a simple dilution or concentration of the index chemical, and, by extension, each other. The joint action of the chemicals, then, can be described by "dose addition" (Finney, 1971).

The fundamental assumption of dose addition is that the components of a mixture exhibit a common toxic mode of action, underlying the addition of scaled doses. Research issues include the development of meaningful toxicological criteria for identifying a common toxic mode of action and the application of these criteria to evaluate and identify mixture components that share a common toxic mode of action.[3]

A theoretical consequence of this assumption is that the dose-response functions of the components exhibit similar shapes. Theoretically, mixture components sharing a common mode of action act as either concentrates or dilutions of each other. The components interact with a common toxicological target, eliciting the same response. Because the chemicals act as concentrates or dilutions of each other, the number of organisms within a dose group responding to the same dose of different chemicals should differ in a consistent manner across doses. The consistent differences in the

[3]There are other mixtures approaches that are based on dose additivity. The hazard index (U.S. EPA, 2000), for example, provides a quantitative method that indicates whether a mixture may pose risk or not. The hazard index method may be used when detailed toxicity data are not available; for example, a hazard index can be developed from exposure estimates and Reference Doses.

responses across dose groups will yield similarly shaped response functions, sometimes referred to as “constant relative potency.” For example, if chemical 2 is one-half as toxic as chemical 1, then, at the same dose, chemical 2 should elicit a response in half as many test organisms as that dose of chemical 1. This pattern should persist with increases of dose until a maximum response is achieved (e.g., 100% response). The similar shapes should also persist as doses are diminished until a response threshold is observed or until one molecule of chemical 2 elicits an observable response. Between the toxicity threshold and the response maximum, similar shapes of the dose-response curves should hold.

In practice, toxicological assays of chemicals having a common mode of action may not exhibit similarly-shaped dose-response functions. Differences in the observed dose-response function shapes between chemicals that share a common mode of action may result from toxicokinetic differences or toxicodynamic differences. Other factors could include differences in age or gender of the animals tested in the bioassay, differences in animal stress status either within or across studies, and differences in whether or not the test animals were naive to the chemical prior to testing. Random errors of response may also explain differences in shape. These random errors describe, from a biostatistical perspective, the distance that an individual’s response may be from the population mean response at a given dose. These differences in the observed dose-response functions may result in different maximal responses as well as different thresholds of response within the exposed population.

These differences in shape of the dose-response functions *may* preclude application of a dose-additive model. If the dose-response functions exhibit different

shapes and the resulting risk estimate predicted by dose addition is quite different from the expected joint mixture response, then scaling the toxicity of one chemical by that of the other may be an inappropriate means of estimating a mixture's risk. The RPF mixture risk model may be rejected under these circumstances, even if the chemicals exhibit a common toxic mode of action.

On the other hand, these differences in shape of the dose-response functions *may not* preclude application of a dose-additive model. If the components exhibit similarly shaped response functions over the relevant range of doses, as judged by the exposure assessment, then the use of dose addition may be valid. This relevant range includes the range of exposures to the individual components and extends to the range of the additive dose (i.e., the total mixture dose in units of the index chemical).

Dose-response modeling research for dose addition includes assessing what is meant statistically by a "similar shape" (see Section 5), including approaches to quantify the amount of uncertainty potentially introduced in the risk estimate when the slopes are dissimilar. Methods and criteria are needed to "determine" when a group of components share a common dose-response function. To conclude, both common mode of toxic action and similarity shaped dose-response functions are prerequisites for valid application of dose addition to a chemical mixture.

3. RELATIVE POTENCY FACTORS

U.S. EPA (2000) developed the RPF approach to assess risks posed by mixtures that are comprised of chemical components exhibiting a common mode of action for a toxic effect. The RPF approach is based on the concept of dose addition. Mixture components are grouped using scientific judgment into subclasses called “RPF Sets” using data on characteristics such as membership in a chemical class (relating to observed toxicity), and commonality of toxicologic effects, exposure routes, exposure durations, or dose ranges. To implement the approach, the exposure level of each component of an RPF Set is scaled by a measure of the component’s toxicity *relative* to a selected index chemical (a toxicologically well-studied component of the RPF Set). This scaling factor, the RPF, is based on a comparison of the component’s toxicity with a similar measure of toxicity for the index chemical (e.g., a ratio of equally effective doses of the component to the index chemical). The product of the measured administered dose of each mixture component and its RPF is defined as an Index Chemical Equivalent Dose (ICED). The ICEDs of all the mixture components are summed to express the total mixture dose in terms of an equivalent dose of the index chemical. The risk posed by the mixture is quantified by comparing a mixture’s total ICED to the dose-response assessment of the index chemical. [The mathematical formulas for the RPF are detailed in Text Box 3-1.]

Appropriate application of the RPF method requires a judgment that the mixture components share a common mode of toxic action and evidence that the components have similarly shaped dose-response curves. Evidence that a chemical class fulfills one of these requirements does *not* necessarily imply that the second requirement is fulfilled. For the first assumption, the term, *Common Mode of Action,* implies that chemicals in a mixture exhibit a common toxicologic outcome when tested and that the principal toxicodynamic events leading to this common outcome after the chemicals reach the target site and the sequence of these events is understood, but many of the details are not known. Because detailed toxicodynamic data are not abundant for most chemical mixtures and their components, analysts typically must judge whether or not the mixture components exhibiting a common toxicologic outcome also share a common mode of action. At times, the term *Common Mechanism*

Text Box 3-1

Mathematical Representations and RPF Formulas

d_1 = dose of chemical 1 present in a mixture (units not specified)

d_2 = dose of chemical 2 present in a mixture (units not specified; must be consistent with those of d_1)

pot_1 = potency estimate (e.g., a slope factor) for chemical 1 (risk per unit of dose specified for d_1)

pot_2 = potency estimate (e.g., a slope factor) for chemical 2 (risk per unit of dose specified for d_2)

ICED = index chemical equivalent dose based on relative potency estimates (units consistent with d_1 and d_2)

$f_1(*)$=dose-response function of the index chemical for the response(s) common to chemical 1 and chemical 2 (units consistent with d_1 and d_2)

$h(d_1,d_2)$ = mixture risk from dose d_1 of chemical 1 and dose d_2 of chemical 2

$[ED_{10}]_1$ = dose of chemical 1 that results in a 10% response, either as a fraction of exposed test animals that respond, or as a fractional change in a measured physiological value.

$[ED_{10}]_2$ = dose of chemical 2 that also results in the same 10% response

Then, designating chemical 1 as the index chemical in the RPF approach,

$RPF_2 = [ED_{10}]_1 / [ED_{10}]_2$,
(or equivalently = pot_2 / pot_1)

$ICED = d_1 + (RPF_2 * d_2)$

$h(d_1,d_2)=f_1(ICED)$ = mixture risk from chemicals 1 and 2 evaluated at the ICED of chemical 1

of Action is used. This term implies a greater degree of understanding of toxicodynamic events, such that the chemicals in a mixture exhibit a common toxicologic outcome when tested and that the underlying molecular and cellular toxicodynamic events leading to this outcome are the same for each chemical, after they reach the target site. (Toxicodynamic events include the initial interaction of a toxicant with its molecular or cellular target and subsequent responses to the toxic insult.) These two terms represent a continuum of toxicodynamic understanding; they are degrees of scientific resolution. For RPFs, there must be a judgment that chemicals exhibiting a common mode of action either do or do not share a common mechanism of action. If judged that they do, then subclasses are not needed. If judged that they do not, then subclasses should be developed and a second set of assumptions should be identified and used to combine (or not combine) the toxicities that the subclasses exhibit.

The second prerequisite for applying an assumption of dose-addition is that the chemicals have similarly shaped dose-response functions at least within the region of exposure of interest for the risk assessment. An evaluation will often be needed of the expected shapes of the dose-response functions in the low dose region *including* the region that may lie below the lowest dose tested in the relevant toxicological bioassay. In Section 5 of this report, we describe procedures that can be used to evaluate similarity among the observable regions of dose-response functions. If there is an evaluation of shape below the experimental response region, it may include an assessment of the mechanism/mode of action.

RPFs are based on comparisons with an index chemical, and the mixture risk is estimated using the dose-response function of the index chemical. Criteria pertaining to

the inclusion of compounds in an RPF Set apply to the index chemical. The index chemical should be a well-studied member of the RPF Set; studies on the index chemical need to provide exposure data for routes of interest and health assessment data for health endpoints of interest. To estimate *relative* potency, toxicity studies of compounds in the RPF Set need to be comparable to studies conducted on the index chemical.

3.1. JUDGMENTS OF COMMON TOXICOLOGIC ACTION

"Pesticides are determined to have a "common mechanism of toxicity" if they act the same way in the body; that is, if scientifically reliable data demonstrate that upon exposure to these chemicals, the same toxic effect occurs in or at the same organ or tissue by essentially the same sequence of major biochemical events" (U.S. EPA, 2002b). The issue of a common mechanism of toxicity has been addressed by a working group of experts convened by the International Life Sciences Institute Risk Science Institute (Mileson et al., 1998).[4] The working group presented three criteria to describe a common mechanism of toxicity: (1) cause the same critical toxic effect; (2) act on the same molecular target at the same target tissue; and (3) act by the same biochemical mechanism of action or share a common toxic intermediate. The working group agreed that all three points are useful to apply to chemicals that may act by a common mechanism of toxicity, but did not state whether all three points must be met before a firm common mechanism of toxicity determination can be reached. It is recognized, however, that precise mechanistic information on animal or human effects

[4]Subsequent to the International Life Sciences Institute expert panel, U.S. EPA issued a guidance document for identifying pesticides with a common mechanism of toxicity (U.S. EPA, 2002b) and a CRA case study for the organophosphorus pesticides (U.S. EPA, 2001b).

for pesticides and most environmental chemicals is scant. Common mechanism determinations will therefore be difficult to establish with these three points because chemicals often exhibit spectra of adverse effects rather than the same critical toxic effect (criterion 1) (Mileson et al., 1998).

Knowledge of a chemical's mode of action implies a general understanding of the key toxicodynamic events that occur at a tissue level, rather than a detailed description of the cascade of events at the cellular or molecular level such as is suggested by the term "mechanism of action." For chemical mixtures, the term, "common mode of action", implies that chemicals exhibit a common toxicologic outcome in the same tissue when tested. However, the toxicodynamic events that lead to this common outcome after the chemicals reach the target site are not well understood; they may be the same (or similar) or not (it is simply not known). A common mode of action is sufficient justification to consider or employ a dose additive model. The terms "mode of action" and "mechanism of action" represent degrees of scientific understanding of toxicodynamic events underlying observed toxic responses rather than separate categories.

The distinction between these two terms is discussed here using a hypothetical cancer assessment to illustrate when dose additive models, such as RPFs, can be used and when they should not be used. (RPFs are relatively simple mixture risk models typically developed from empirical bases; as additional detailed toxicodynamic data are generated for mixture components, these simple models are likely to be replaced by biologically-based mixture risk models.) Tumors occurring in a specific liver tissue in an

animal bioassay may arise through a number of different modes of action. Consider two examples.

Example Chemical 1:

Repeated episodes of chemical-induced liver cell necrosis may result in random DNA replication errors as the surviving cells undergo compensatory reproduction. These random DNA replication errors may occur in genes critical to control of cell replication (e.g., tumor suppressor genes and proto-oncogenes) and become "fixed" in the genome through replication, ultimately giving rise to liver tumors.

Example Chemical 2:

A mutagen may interact directly with liver cell DNA that codes for genes in the cell replication cycle and cause a mutation that gets fixed in the DNA after a round of replication. Some of these mutations reduce the cells' ability to properly regulate their own replication and this lack of replicative control ultimately results in tumor development after a series of additional mutations and changes occur in the affected cells.

These two chemicals do not share a common mechanism of action because the first induces carcinogenesis through necrosis and the second induces carcinogenesis through mutation of the target tissue.

Now, assume that two chemicals that comprise an environmental mixture both cause necrosis in the same hepatic tissue when tested individually in separate animal bioassays. The ultimate result of the liver tissue necrosis that occurs when each chemical is tested in a bioassay is the formation of observable liver tumors in the same tissues (as in Example Chemical 1 in the preceding paragraph, the tumors form when

random errors in DNA replication occur in genes that control the cell cycle get “fixed” during compensatory liver cell replication). The same bioassay outcome (i.e., liver tumor formation arising from a specific tissue when each individual chemical is tested) may occur through a number of different possible necrotic processes that lead to dead liver cells:

1) The chemicals may cause liver tissue cell necrosis by the same mechanism of toxic action. The chemicals may be shown to cause the same sequence of necrotic events in affected liver cells after the chemicals reach the target tissue. This is clearly a case of chemicals exhibiting a *common mechanism of action*. Lacking the level of mechanistic detail provided in this description, analysts could still logically conclude that the chemicals may share a *common mode of action* based on the occurrence of liver tumors arising in the same hepatic tissue.

2) The chemicals may cause tissue necrosis in the same liver cells by different necrotic mechanisms (i.e., either different toxicodynamic events or different sequences of toxicodynamic events that are observed to lead to cell death...ultimately resulting in tumor formation through random errors in compensatory replication in remaining living cells). In this case, one could reasonably judge that the chemicals still exhibit a *common mechanism of action*. Lacking the mechanistic detail, analysts could still logically judge that the chemicals share a *common mode of action* because of the occurrence of liver tumors arising in the same hepatic tissue*s.*

For the two cases above, RPFs for the chemicals could be developed (given appropriate data).

3) If the chemicals cause tumors in different types of cells of the same organ, then, based on evidence from animal bioassays, it is concluded that the chemicals do *not* share a *common mode of action*. Because they are not causing necrosis in the same types of cells, it could be concluded that the chemicals cause toxicity through different modes of action.This outcome could occur because of toxicokinetic differences between the chemicals, toxicodynamic differences between the chemicals, or both. In any case, it is not appropriate to use RPFs for the assessment of risk posed by this mixture, based on the available toxicodynamic information.[5]

[5]In practice, U.S. EPA (2000) suggests use of the Hazard Index (HI) method as an indicator of risk when mixture components cause toxicity in the same target organ. In this case dose addition is loosely defined to accomodate the lack of accessible mechanistic data.

4. CHOICE OF DOSE METRIC IN CHARACTERIZING MIXTURE TOXICITY BY DOSE ADDITION

Two separate issues are discussed in this section. First, the potential significance of using kinetic data in the development of relative potency factors is described. If kinetic differences exist between test species and humans, relative potency factors will change when modeling risks on the basis of a administered dose versus an internal dose. Second, a discussion is presented regarding choice of dose metric in an RPF-based approach. In modeling human health risks posed by exposure to a mixture by the RPF method, the type of dose measures employed do not appear to alter the outcome of the risk estimation procedure. Two dose measures commonly used for delivered dose are units of mass (mg/kg) or moles (mmol/kg). The key is to be consistent in development of an RPF application, using either mass measures or molar measures.

4.1. RPF DOSE ISSUES

Measures of either an administered dose or internal dose may provide the basis for estimation of relative potency for a chemical group. Administered or applied doses are the amount of a substance applied to an external body barrier and available for absorption. Administered doses include those doses applied to external body membranes such as the gastrointestinal tract, the lungs and the skin. Internal doses measure or estimate the quantity of a contaminant that is present in an internal tissue (U.S. EPA, 1992). The entire administered dose may not cross the barrier. Tissue concentrations of interest could include those occurring at either toxicologic target tissues and or tissues not targeted by the chemical.

4.1.1. Administered Dose. Most applications of RPFs in the literature are based on measures of administered doses. For example, the EPA has developed four sets of RPFs that estimate the toxicity of a mixture of related compounds based upon administered dose measures for individual compounds: the dioxins, the polychlorinated biphenyls, the polycyclic aromatic hydrocarbons, and the organophosphorus pesticides (OP) (U.S. EPA, 2000, 2001b). In each case, the risk estimates based on RPFs were described as interim, pending the emergence of additional chemical mixture-specific toxicokinetic and toxicodynamic data. The type of dose upon which the RPFs are based will not alter the interim nature of the risk estimate. Ultimately, biologically-based mixtures risk models will likely be developed for each case; these models will replace the simpler RPF models and be based upon the emergence of additional chemical mixture-specific toxicokinetic and toxicodynamic data.

4.1.2. Internal Dose. Measures or estimates of internal doses may provide an improved basis both for estimating risks posed by chemical mixtures that occur through multiple exposure routes and for estimating human health risks for some mixtures by the same exposure route. To date, RPFs based on internal doses have not been developed because the ability to predict internal organ or tissue doses through physiologically-based pharmacokinetic (PBPK) models is relatively new or because, given the simplistic assumptions of the RPF approach, refined estimates of dose would provide little resolution to overall uncertainty.

4.1.3. Mixtures Exposures Through Multiple Exposure Routes. In 2002, U.S. EPA completed a report showing that a multiple exposure route mixtures risk assessment can be conducted based on internal dose estimates developed in both test animals and

humans for toxicants that do not cause portal of entry effects (U.S. EPA, 2002c). The document combines exposure modeling results, PBPK modeling results, and the RPF mixtures risk assessment approach. Human internal doses (e.g., blood, tissue, and organ concentrations) were estimated using PBPK models, accounting for external exposures from multiple routes (as dictated by the exposure scenario) and human PK processes. Hypothetical RPFs were developed for a subset of chemicals based on test animal data. Although the application of a full PBPK model was recognized as the preferred approach to estimating rodent internal doses (i.e., blood concentrations), for the example data used in the report, administered doses were assumed to be 100% bioavailable to the rat. The rodent response data were assumed to be constant between internal and external exposures and were used to evaluate the human dose-response relationship. The use of internal dose measures (i.e., blood concentrations in both humans and rodents) both for developing the RPFs based on rodent data and as an indicator of human multi-route exposure provides a necessary and consistent basis for extrapolating across species. Clearly, these approaches should not be used and are inappropriate for toxicants that elicit responses at points of contact with the body (e.g., skin, intestinal tract, and nasopharyngeal, bronchial and lung epithelium).

4.1.4. Mixtures Exposures Through a Single Exposure Route in Different Species. For some mixtures, basing RPFs on internal doses may reduce some uncertainty in applying RPFs for individual exposure routes. From a single route of exposure to a given chemical mixture, the animal kinetics and human kinetics that give rise to respective internal doses of the mixture components may result either in the same internal doses or different internal doses, when the same amount of chemical is applied

externally. If the kinetics result in the same internal doses or internal doses that differ consistently across the mixture (i.e., comparisons of the ratios of external to internal doses for each component between test animal and human are constant), then basing RPFs on internal dose estimates is not necessary, because the relative potencies will not change. When kinetic differences between humans and test animals lead to non-constant differences in internal dose concentrations across a chemical class, then basing RPFs on internal doses provides a more scientifically sound basis for applying RPFs (see Text Boxes 4-1 and 4-2).

Consider the same 2 component mixture example presented in Text Box 3-1 where chemical 1 again serves as the index chemical. Rodent data exist

Text Box 4-1

Mathematical Representations and Formulas for RPF Based on Internal Doses to Rats (test animal)

Let:

d_1 = exposure to chemical 1 as a result of its presence in a mixture (units not specified)

d_2 = exposure to chemical 2 as a result of its presence in a mixture (units not specified; consistent with d_1)

I_1 = internal dose of chemical 1 present in a mixture (units not specified)

I_2 = internal dose of chemical 2 present in a mixture (units not specified; consistent with I_1)

$ICED_I$ = index chemical equivalent dose based on relative potency estimates (units consistent for I_1 , I_2)

$f_1(*)$=dose-response function of the index chemical for response(s) common to chemicals 1 and 2 (units consistent with I_1 and I_2; they are based on internal measures of dose but use the same response measures as developed in Text Box 3-1)

$h(I_1,I_2)$ = mixture hazard or risk from joint exposure of dose d_1 to chemical 1 and dose d_2 to chemical 2; however, these doses are based on internal measures I_1 and I_2 rather than administered doses d_1 and d_2.

$[ED_{10}]_{I1}$ = internal dose of chemical 1 that results in a 10% response, either as a fraction of exposed test animals that respond, or as a fractional change in a measured physiological value.

$[ED_{10}]_{I2}$ = internal dose of chemical 2 that also results in the same 10% response
Then, designating chemical 1 as the index chemical in the internal dose based RPF approach,

$RPF_{2I} = [ED_{10}]_{I1} / [ED_{10}]_{I2}$

$ICED_I = I_1 + (RPF_2 * I_2)$

$h(I_1,I_2)=f_1(ICED_I)$

such that a RPF_2 can be developed based on the administered doses (RPF_{2E}) or the internal doses (RPF_{2I}) of chemical 1 and chemical 2. Response data are available for the test animals only, and the human mode of action is considered to be the same as that in the test animal. The ratio, RPF_{2E}/RPF_{2I}, in the rodent will either approximately equal the ratio of human administered dose to human internal dose or not. If these ratios are equal, then, when estimating risk using the RPF approach for a single exposure route, it does not matter whether external or internal doses are used as the basis of the RPF. If these ratios are not equal, then, when estimating risk using the RPF approach for a single exposure route, it matters whether external or internal doses are used as the basis of the RPF. The RPFs should be based on internal doses because the

Text Box 4-2

Potential Use Of Internal Dose Based RPFs

Assume that the toxicodynamics are the same for humans and rats. Let:
$K_{1R}= I_1/d_1$ in Rat for chemical 1
$K_{2R}= I_2/d_2$,in Rat for chemical 2
$K_{1H}= I_1/d_1$ in Human for chemical 1
$K_{2H}= I_2/d_2$,in Human for chemical 2
From Text Box 3-1, the mixture risk in rats is $h(d_1,d_2)=f_1(ICED)$
This is based upon $ICED = d_1 + (RPF_2 * d_2)$, where $RPF_2 = [ED_{10}]_1 / [ED_{10}]_2$.
An implicit assumption in the Chemical Mixture Guidance is that RPF_2 is the same in rodents and humans. Thus, the human ICED for d_2 is calculated as the product of the human administered dose and RPF_2.
The risk posed to humans from this mixture is estimated to be $h(d_1,d_2)=f_1(ICED)$,
where $ICED = d_1 + (RPF_2 * d_2)$ and $RPF_2 = [ED_{10}]_1 / [ED_{10}]_2$. The ratio of $[ED_{10}]_s$ is calculated from the rodent administered dose data.
Proposal:If $K_{1R} = K_{1H}$ and $K_{2R} \neq K_{2H} \Rightarrow RPF_2$ is ***not*** a valid estimate of the relative potency of chemical 2 for the human.
Proof: Let chemical 2 be converted to chemical 1 on a 2 to 1 molar basis in the rat (i.e., 2 moles of chemical 2 is converted thru some kinetic process into 1 mole of chemical 1 in the rat). For an RPF model, chemical 2 would be one-half as toxic relative to chemical 1 based on the administered doses $\Rightarrow RPF_2 = 0.5$, when chemical 1 is the index chemical and $RPF_1 = 1$.
Let the conversion of chemical 2 to chemical 1 cause toxicity of chemical 2 in the human also and assume that the toxicodynamics of chemical 1 are identical for humans and rats.
Because $K_{2R} \neq K_{2H}$, the conversion of chemical 2 into chemical 1 will *not* exhibit a 2 to 1 ratio, the RPF_2 estimated from rodent external data $\neq$ the human RPF_2. The kinetic differences between humans and rodents lead to different internal tissue doses which influence the toxicity of chemical 2 *relative* to chemical 1.
Further Implication
If $K_{1R}/K_{1H} = K_{2R}/K_{2H} \Rightarrow$ It is valid to apply RPF_2 estimated from rat data to human administered dose data due to kinetic differences. The kinetic differences between species do not change the *relative* potency of Chemical 2 to Chemical 1.

pharmacokinetic differences result in inaccuracies when the RPFs are developed in test animals and applied to humans.

4.2. CHOICE OF DOSE MEASURES

For modeling of a mixture's toxicity or joint action under the assumptions of relative potency factors, representation of dose as either a molecular (molar) representation of dose or a representation by chemical mass does not matter in the conduct of the risk assessment. It does not matter because *the molecular weights of the compounds relative to each other* are constant.

Consider two compounds, C1 and C2, that exhibit a common mechanism of toxic action. Let the molecular weight of C2 be twice that of C1. Administration of 1 milligram of C1 elicits the same response in test animals as administration of 2 milligrams of C2. (Molecules of C1 and C2 are equally potent.) If the experimental evidence for RPFs is based on single chemical experiments where dose is measured in milligrams, then the relative potency of C2 to C1 will be 0.5. If the experimental evidence for RPFs is based on single chemical experiments where dose is measured in moles, then the relative potency of C2 to C1 will be 1. Because a molecule of C2 has twice the mass of C1, the conversion of mass doses to molar doses in a risk assessment will result in an RPF for C2 of 0.5 (i.e., = ½). Equivalent human exposures (resulting in the same predicted risk) result from exposures to 1 mole of each chemical or some mass of C1 and ½ the same mass of C2. Thus, the chemical potency comparisons when applied to estimate human risk will be the same regardless of whether the measures are based on moles or masses.

5. BIOSTATISTICAL DOSE-RESPONSE MODELING FOR CUMULATIVE RISK

Biostatistical modeling results can be integrated with exposures to calculate cumulative risk estimates depending on expected toxicological action of the mixture components. Three methods discussed in this section:

1) *Dose Addition:* When the chemicals of interest act in accordance with a common mode of action, a dose addition approach can be employed. Dose Addition is a chemical mixtures risk assessment method in which doses are summed (after scaling for relative potency) across chemicals that have a similar mode of action; risk is then estimated using the combined total dose.

2) Integration of Dose Addition and Response Addition: When mixture components can be classified into subgroups within which a common mode of action exists, then, by definition, independence of toxic action is expected between subgroups. Response addition is a chemical mixtures risk assessment method applied to chemicals whose modes of action are independent of each other (i.e., the presence of one chemical in the body does not influence the effects caused by another chemical); risk of a whole body effect (e.g., non-specific cancer), is then estimated by summing the risks (e.g., skin cancer, liver cancer) of the individual chemicals. Integrating dose addition and response addition in this case means to estimate the subgroup risks and then sum them to estimate cumulative risks (U.S. EPA, 2002c).

3) *Joint Dose-Response Model:* Finally, a joint dose-response model using scaled doses is applied when commonality of toxic mode of action is uncertain. This method produces a range of cumulative risk estimates.

EPA-sponsored research on the use of dose-addition in cumulative risk assessment, focusing on the issue of similarly shaped component dose-response curves, has resulted in the publication of two papers by Chen et al. (2001, 2003). The information in this chapter relies heavily on the research presented in the Chen et al. papers, which are reproduced in their entirety in Appendices A and B. The first paper (Chen et al., 2001) demonstrates methods for dichotomous data using the log probit and logistic dose-response functions. The second paper (Chen et al., 2003) further extends the statistical methods to continuous endpoints, using cholinesterase inhibition as an example. To demonstrate use of these models in cumulative risk assessment, without loss of generality, the discussions in this section are limited to dichotomous data using the log probit dose-response function.

5.1. DOSE–RESPONSE MODEL FOR COMBINED EXPOSURES

To begin discussion of dose-addition as a tool for risk assessment, let F_1 and F_2 be the dose-response functions for chemical 1 and chemical 2, respectively. Under dose addition, the response, R, to the combination of doses d_1 and d_2 for chemicals 1 and 2, respectively, is

$$R(d_1, d_2) = F_1(d_1 + \rho\ d_2) = F_2(d_1/\rho\ + d_2) \qquad (5\text{-}1)$$

where ρ is the relative potency of chemical 2 to chemical 1. When one chemical acts as if it is a simple dilution or concentration of the other, then the relative potency between the two chemicals is constant. In other words, for all response levels, the effective dose

of one chemical is a constant multiple of the effective dose of the other chemical. Hewlett and Plackett (1959) viewed the concept of dose addition (similar action) in a slightly broader sense than requiring a constant relative potency between two chemicals. Mathematically, their characterization can be interpreted as allowing the relative potency factor to be different for different response levels. Thus, the biological bases and mathematical models required to characterize an RPF-based assessment are different depending on whether or not constant relative potency is assumed.

Dose addition allows for summing the individual doses into an equivalent dose in terms of an index chemical and using the index chemical's dose-response function to estimate the mixture response from the equivalent total mixture dose. A dose-response function for binary response data, denoted $P_i(d) = F$, relates the probability of response to the dose, d, of chemical i, where F is a probability distribution function. The general model can be expressed in the logarithm of dose as

$$P_i(d) = F\left(\alpha_i + \beta_i log d\right) \tag{5-2}$$

A commonly used dose-response model, used throughout this disscussion to illustrate the methods, is the probit function, which is,

$$P(d) = c + (1 - c)\int_{-\infty}^{\alpha+\beta log d} \frac{1}{\sqrt{2\pi}} exp\left(-1/2\, t^2\right) dt \tag{5-3}$$

where the parameter c represents background effect and P(d) is defined to be c when d = 0. The parameters α and β are the intercept and slope parameters of the dose-response function under its inverse, $F^{-1}(P(d))$. *For the rest of this discussion, the log probit function for binary data will be used to demonstrate dose addition methods;*

however, other functions and continuous endpoints can also be utilized in these approaches (see Appendices A and B).

For an example with two chemicals, if the relative potency of chemical 2 to chemical 1 is constant, then the dose-response for one chemical can be expressed in terms of the equivalent dose of the other chemical by using a relative potency factor. In this case, $\rho = (d_1 / d_2)$ (i.e., $P_1(d_1) = P_2(d_1 / \rho) = P_1(\rho d_2) = P_2(d_2)$), where the dose d_1 of chemical 1 and d_2 of chemical 2 are equal effective doses (i.e., they cause the same magnitude of response). Now, given that $P_1(d_1) = P_2(d_1/\rho)$, then

$$\alpha_1 + \beta_1 \log d_1 = \alpha_2 + \beta_2 \log\left(d_1 / \rho\right) \tag{5-4}$$

The above equality holds for all doses of chemical 1, d_1. To simplify, then, let $d_1 = 1$, and the equation, that holds true for all doses, becomes

$$\alpha_1 = \alpha_2 - \beta_2 \log \rho \tag{5-5}$$

This implies that

$$\log \rho = \left(\alpha_2 - \alpha_1\right) \Big/ \beta_2 \tag{5-6}$$

Repeating the process for $P_2(d_2) = P_1(\rho d_2)$, then, analogously, we get,

$$\log \rho = \left(\alpha_2 - \alpha_1\right) \Big/ \beta_1 \tag{5-7}$$

Hence, because Equations 5-6 and 5-7 are both true, $\beta_1 = \beta_2$, and it can be shown that two chemicals have a constant relative potency if and only if the slopes of the (log) dose-response functions are equal. (See Appendix A for a more complete proof.)

5.1.1. Constant Relative Potency. The term, constant relative potency, implies that for all response levels, the effective dose of one chemical is a constant multiple of the effective dose of the other chemical. Constant relative potency is a desired condition to conduct an RPF based risk assessment, at least for the dose ranges pertinent to the exposure of interest (see Section 2).

5.1.1.1. Dose Addition — If two chemicals have a constant relative potency and if the joint response is dose-additive, then the dose-response function from exposure to d_1 of chemical 1 and d_2 of chemical 2, using chemical 1 as the index chemical is,

$$F(d_1, d_2) = P_1(d_1 + \rho\, d_2) = F(\alpha_1 + \beta\ \log(d_1 + \rho\, d_2)) \tag{5-8}$$

For a group of *m* chemicals in which the relative potency between any two chemicals is constant, the joint response of the *m* chemicals can be derived in the same way as Equation 5-8, using a relative potency factor ρ_t for each component as it is paired with the index chemical(s).

$$F(d_1, \ldots d_m) = F\left[\alpha_s + \beta\ \log\left(d_s + \sum_{t \neq s}^{m} \rho_t d_t\right)\right] \tag{5-9}$$

where, $\rho_t = \exp[\,(\alpha_t - \alpha_s) / \beta\,]$ for $t \neq s$. In this case, the estimated risk at any set of doses does not depend on the choice of index chemical (i.e., when constant relative

potency is operational, the risk estimate will be the same regardless of the choice of index chemical).

5.1.2. Nonconstant Relative Potency. Constant relative potency is a fairly restrictive assumption that may not hold true for many mixtures. Thus, if the relative potency between chemical 1 and chemical 2 is different for different response levels, then the slopes of the dose-response functions for the two chemicals will be different and the modes of action for the two chemicals may also differ. In this case, at the equal effective doses of d_1 for chemical 1 and d_2 for chemical 2 such that $P_1(d_1) = P_2(d_2)$, it can be shown that the equivalent dose of chemical 2 in terms of chemical 1 is,

$$d_1(d_2) = \exp\left(\frac{\alpha_2 - \alpha_1}{\beta_1}\right) d_2^{\beta_2/\beta_1} \tag{5-10}$$

and the equivalent dose of chemical 1 in terms of chemical 2 is,

$$d_2(d_1) = \exp\left(\frac{\alpha_1 - \alpha_2}{\beta_2}\right) d_1^{\beta_1/\beta_2} \tag{5-11}$$

Under these conditions, the joint response can still be estimated by an index chemical approach, using doses adjusted by a ratio of the slopes. The joint dose-response from an exposure to d_1 of chemical 1 and d_2 of chemical 2 in terms of chemical 1 as the index chemical is,

$$F(d_1, d_2) = P_1(d_1 + d_1(d_2)) = F(\alpha_1 + \beta_1 \log(d_1 + \rho_{12} d_2^w)) \tag{5-12}$$

where w = β_2 / β_1, and $\rho_{12} = \exp[(\alpha_2 - \alpha_1) / \beta_1]$. On the other hand, the joint response in terms of chemical 2 as the index chemical is,

$$F(d_1, d_2) = P_2(d_2 + d_2(d_1)) = F(\alpha_2 + \beta_2 \log(d_2 + \rho_{21} d_2^{1/w})) \quad (5\text{-}13)$$

where w = β_2 / β_1, and $\rho_{21} = \exp[(\alpha_1 - \alpha_2) / \beta_2]$. Note that the joint response predicted from chemical 1, $P_1(d_1 + d_1(d_2))$, will differ from that predicted from chemical 2, $P_2(d_2 + d_2(d_1))$. For *m* chemicals, the combined response in terms of chemical *s* can be derived as,

$$F(d_1, \ldots, d_m) = P_s\left(d_s + \sum_{t \neq s}^{m} \rho_{st} d_t^{w_{st}}\right) = F\left(\alpha_s + \beta_s \log\left(\sum_{t \neq s}^{m} \rho_{st} d_t^{w_{st}}\right)\right) \quad (5\text{-}14)$$

The $\rho_{st} = \exp[(\alpha_t - \alpha_s) / \beta_s]$ is a potency ratio of chemical t to the index chemical s, and $w_{st} = \beta_t / \beta_s$, is the slope ratio, where t = 1,...,m, and $t \neq s$.

5.1.3. Constant and Nonconstant Relative Potencies in the Same Mixture. In many cases, a mixture may be comprised of component subsets, where within each subset a constant relative potency may exist (dose addition for common modes of action), but where nonconstant relative potencies occur between subsets (response addition for independence of action between subsets). In this case, a set of *m* chemicals can be clustered into several subclasses of constant relative potency. For example, the set of six chemicals,

{{C1,C2,C3},{C4,C5},{C6}},

represents a set where the chemicals C1, C2, and C3 in the first subclass have constant relative potency with respect to each other, as do the chemicals C4 and C5 in

the second subclass; the relative potency factor between the last chemical C6 and the other chemicals is different at different response levels. Two approaches are proposed here for evaluating a set of chemicals with varying relative potencies.

5.1.3.1. Integrating Dose Addition and Response Addition — The first approach is appropriate to apply when data on the toxic modes of action are available so there is some certainty that the subclasses represent groups of chemicals with a common mode of action distinctly different from the other subclasses. The toxicity associated with each subclass is produced independently from the other subclasses. The statistical method is then to estimate the dose-response function for the chemicals within each subclass under dose addition, using a different index chemical from within each subclass, and calculate the joint cumulative risk under response addition as the sum of the subclass risk estimates. Hence, the joint dose-response function is expressed as,

$$F(d_1,\ldots d_m) = P_1\left[d_1 + \rho_{12}d_2 + \ldots\right] + \ldots + P_q\left[d_q + \rho_{q,q+1}d_{q+1} + \ldots\right] + \ldots P_m\left[d_m\right] \qquad (5\text{-}15)$$

where ρ_{st} is the relative potency factor for chemical s and index chemical t. In this case the risk estimate would be made using a unique index chemical for each subclass; the risk estimates for each subclass would be summed using response addition. (The complete derivation for Equation 5-15 can be found in Appendix B.)

5.1.3.2. Joint Dose Response Modeling to Reflect Uncertainty of Mode of Action — The second approach is applied when the toxic modes of action for the components are more uncertain. In this case, it is proposed that a range of risk estimates be produced, repeating the risk calculations several times, each time

selecting an index chemical from a separate subclass. Based on Equation 5-14 developed above, the joint response for *m* chemicals can be expressed in terms of a single index chemical 1 for the entire mixture (i.e., including all subclasses) as,

$$F\left(d_1,\ldots d_m\right) = P_1\left[\left(d_1 + \rho_2 d_2 + \ldots\right) + \ldots + \left(\rho_l d_l^{wl} + \rho_{l+1} d_{l+1}^{wl} + \ldots\right) + \ldots + \rho_m d_m^{wm}\right] \qquad (5\text{-}16)$$

The chemicals in the same subclass will have the same slope ratio $w_t = (\beta_t / \beta_1)$. Also, the chemicals within the same subclass will have the same cumulative risk estimate, regardless of the choice of index chemical. (The complete derivation for Equation 5-16 can be found in Appendix A.) However, the estimated combined response will depend on the subclass in which the index chemical is selected, a different subclass will predict a different risk estimate. Thus a range of risk estimates can be produced, reflecting the uncertainty in the mode of action determinations.

5.2. STATISTICAL ALGORITHMS FOR SUBCLASS GROUPINGS WITHIN A MIXTURE

Two classification algorithms are proposed to cluster mixture components into subclasses such that the chemicals in the same subclass have a common slope. The joint response is estimated by fitting the dose-response model of the mixture under dose addition. Chemicals within subclasses are first combined using simple dose addition (constant relative potency), and then subclasses of chemicals are combined using a general form of dose addition (non-constant relative potency). Thus, the proposed method allows one to estimate the joint toxic response for chemicals having different dose-response slopes. (A complete example of the classification algorithms and subsequent response calculations for six hypothetical pesticides in a mixture are shown in Section 4 of Appendix B.)

Since two chemicals have a constant relative potency if and only if the slopes of the (log) dose-response functions are equal, the clustering algorithm is based on testing for the equality of the slopes of dose-response functions. Either the likelihood ratio test or the analysis of variance *F* test can be used for the comparison. (See Appendix B for more information on these tests.) The clustering algorithms begin with a fitting of each individual dose-response function for the *m* chemicals. Let, $\hat{\beta}_1, \hat{\beta}_2, \ldots \hat{\beta}_m$ denote the maximum likelihood estimates of the dose-response functions. The estimates of the *m* slopes can be arranged in an ascending order:

$$\hat{\beta}_{c1} \leq \hat{\beta}_{c2} \leq \ldots \leq \hat{\beta}_{cm}.$$

That is, the chemical c_1 has the smallest slope estimate, the chemical c_2 has the second smallest slope estimate, and so on. The classification algorithms are applied to this ordered set. These iterative (stepwise) processes systematically test the adjacent chemicals in an ordered set for equal slopes and end up with subclasses of chemicals that can be characterized as having the same slope. The top-down approach begins with the assumption that all of the slopes are different and uses an iterative process to group chemicals with common slopes into subclasses; the bottom-up approach begins with the assumption that all of the slopes are equal and uses an iterative process to divide the chemicals into subclasses that have different slopes.

In classical statistics, when the null hypothesis is rejected, this result does not imply that the null is then true and can be accepted. For example, in the bottom-up approach, the procedure keeps dividing the chemicals into RPF subclasses until the null hypothesis is not rejected. We complete the procedure when we can accept the null

hypothesis that dose-response slopes among chemicals are the same. In the top-down approach, the procedure keeps grouping the chemicals until the null model is rejected. We complete the procedure when we can reject the null hypothesis that dose-response slopes among chemicals are the same. Thus, the top-down approach may be a preferred method because there, the procedure is more consistent with traditional statistics. For this application, however, useful information is gained using either approach regarding how different two or more curves might be, offering a quantitative method to assess similarity in dose-response beyond the more typical visual check using graphics. Because we want to "travel up" the dose-response curve of the index chemical to predict mixture risk, we need some comfort level that the dose-response curves of the subclass chemicals share a common shape.

5.2.1. Top-Down Approach. In the top-down classification, the procedure begins using an initial model in which the slopes of the *m* chemicals are assumed to all be different. Figure 5-1 illustrates the iterative procedure followed using the top-down approach. (See also Table 3 in Appendix B for example calculations.) The initial model, M0, of chemicals is denoted by the partition set M0 = {{C1}, {C2}, {C3}, {C4}, {C5}, {C6}}. Consider the null and alternative hypotheses, comparing two adjacent slopes,

$$H_{oq}: \beta_{c,q} = \beta_{c,q+1} \quad \text{versus} \quad H_{aq}: \beta_{c,q} \neq \beta_{c,q+1} \tag{5-17}$$

for q = 1, 2 ,..., m-1. Under the null hypothesis, a joint dose-response function can be fit for the mixture of chemicals C_q and C_{q+1}, using a constant relative potency model, based on Equation (5-8) of,

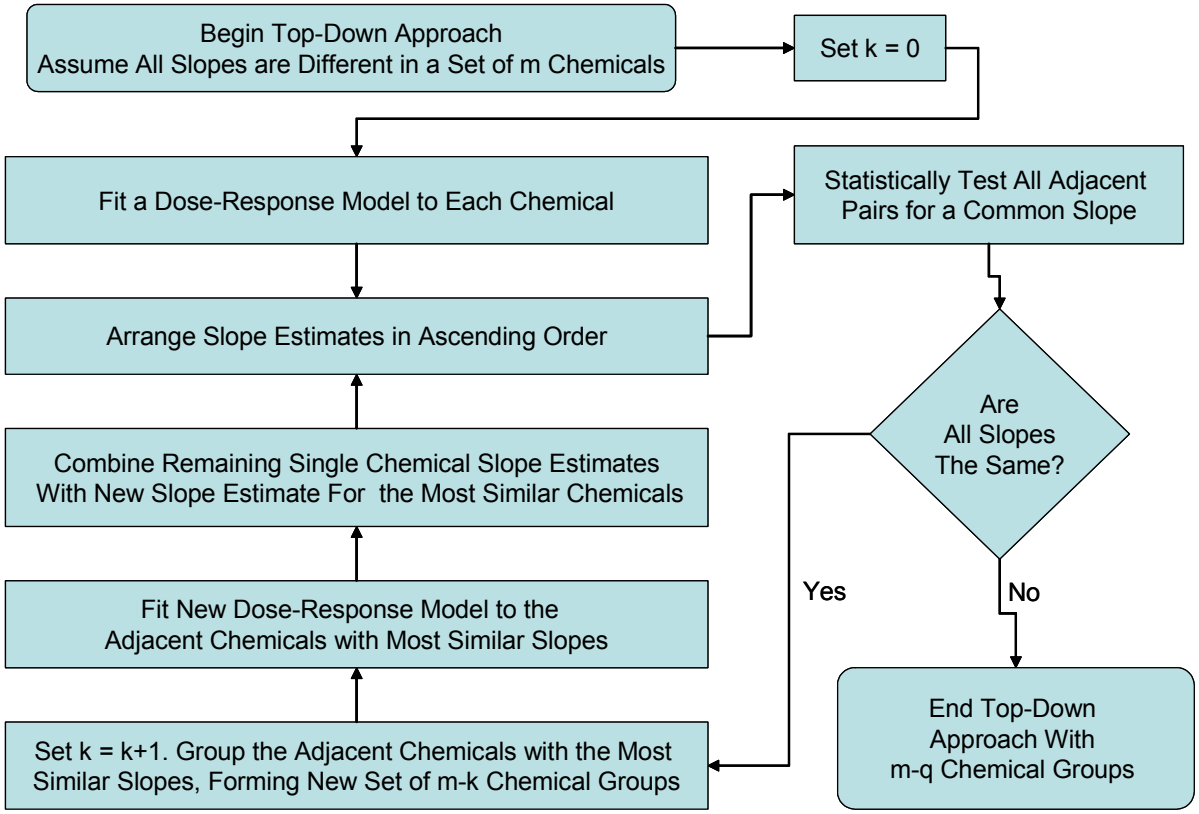

FIGURE 5-1

Flow Chart for Top-Down Approach

$$F\left(d_q, d_{q+1}\right) = P_q\left(d_q + \rho_{cq,cq+1}\, d_{q+1}\right) = F\left(\alpha_{cq} + \beta_{cq} \log\left(d_q + \rho_{cq,cq+1}\, d_{q+1}\right)\right) \quad (5\text{-}18)$$

The null model H_{0q} can be represented by the partition set,

$$B1 = \{\{C1\}, \ldots \{C_q = C_{q+1}\}, \ldots \{Cm\}\}. \quad (5\text{-}19)$$

The hypothesis of comparing two adjacent slopes, equivalently, can be expressed in terms of testing the two models, the null model (B1) vs. the initial model (M0):

H_{oq}: B1 = {{C1}, ... $\{C_q = C_{q+1}\}$, ... {Cm}} versus H_{aq}: M0 = {{C1}, {C2}, ... , {Cm}}.

Let p_{oq} be the p-value associated with the test H_{oq} versus H_{aq}, for q = 1, 2 ,..., m-1; and let p_{cr} = Max { $p_{c1,}\, p_{c2,\ldots} p_{cm}$} (i.e., p_{cr} is the largest p value associated with testing for a common slope between two adjacent chemicals in the set). When the largest value, p_{cr}, is less than a pre-specified significance level, say, α_k then the procedure stops, we reject the null model that the chemicals can be further grouped, and the model M0 that the slopes of the *m* chemicals are different is concluded. On the other hand, if p_{cr} is greater than the significance level, then we cannot reject the null model, so the chemicals C_r and C_{r+1} are classified into one subclass. That is, a new "initial" model, M1 = {{C1}, ... $\{C_q = C_{q+1}\}$, ... {Cm}}, is formed and the procedure continues to the next step.

Under the model M1, the two chemicals c_r and c_{r+1} can be treated as one chemical. Let, $\hat{\beta}'_{cr}$ denote the maximum likelihood estimate of the common slope for the two chemicals c_r and c_{r+1}. The *m-1* slope estimates are now arranged in ascending order as:

$$\hat{\beta}_{c1} \leq \ldots \hat{\beta}_{cr-1} \leq \hat{\beta}'_{cr} \leq \hat{\beta}_{cr+2} \ldots \leq \hat{\beta}_{cm}.$$

That is, the two individual slope estimates, $\hat{\beta}_{cr}$ *and* $\hat{\beta}_{cr+1}$ are replaced by their common slope estimate, $\hat{\beta}_{cr}^{'}$. The same algorithm is applied by performing *m-2* comparisons of two adjacent estimates. The hypothesis can be expressed as

H_{0q} : B2 versus H_{aq} : M1

where

B2 = { ..., $\{C_q, C_{q+1}\}$, ... , $\{C_r, C_{r+1}\}$, ...} if $q \neq (r - 1)$ or $q \neq (r + 1)$

= { ..., $\{C_{r-1}, C_r, C_{r+1}\}$, ...} if $q = (r - 1)$

= { ..., $\{C_r, C_{r+1}, C_{r+2}\}$, ...} if $q = (r + 1)$

Again, if the largest p-value is less than the significance level, then the procedure stops, the null hypothesis that the slopes are the same is rejected, and the model M1 is concluded. If the largest p-value is greater than the significance level, then the null model is adopted as a new "initial" model and the procedure continues to the next step. The procedure keeps grouping the chemicals until the null model is rejected. Note that in the last step, if the null hypothesis is not rejected, then the model {C1, C2,...,Cm}, that all slopes are equal, is used for the risk assessment.

5.2.2. Bottom-Up Approach. In the bottom-up classification, the procedure starts with the initial model, MO, where the slopes of the *m* chemicals are equal, denoted as the partition set, M0 = {C1, C2,...,Cm}. (The same notation is used to illustrate the parallelism between the two classification schemes.) Figure 5-2 illustrates the iterative procedure followed using the bottom-up approach. (See also Table 4 in Appendix B for example calculations.) We now form a new model B1 = {{C1, ... C_q}, $\{C_{q+1}$, ... , Cm}} constructed by the split of M0 into two subclasses. Consider the hypothesis of a

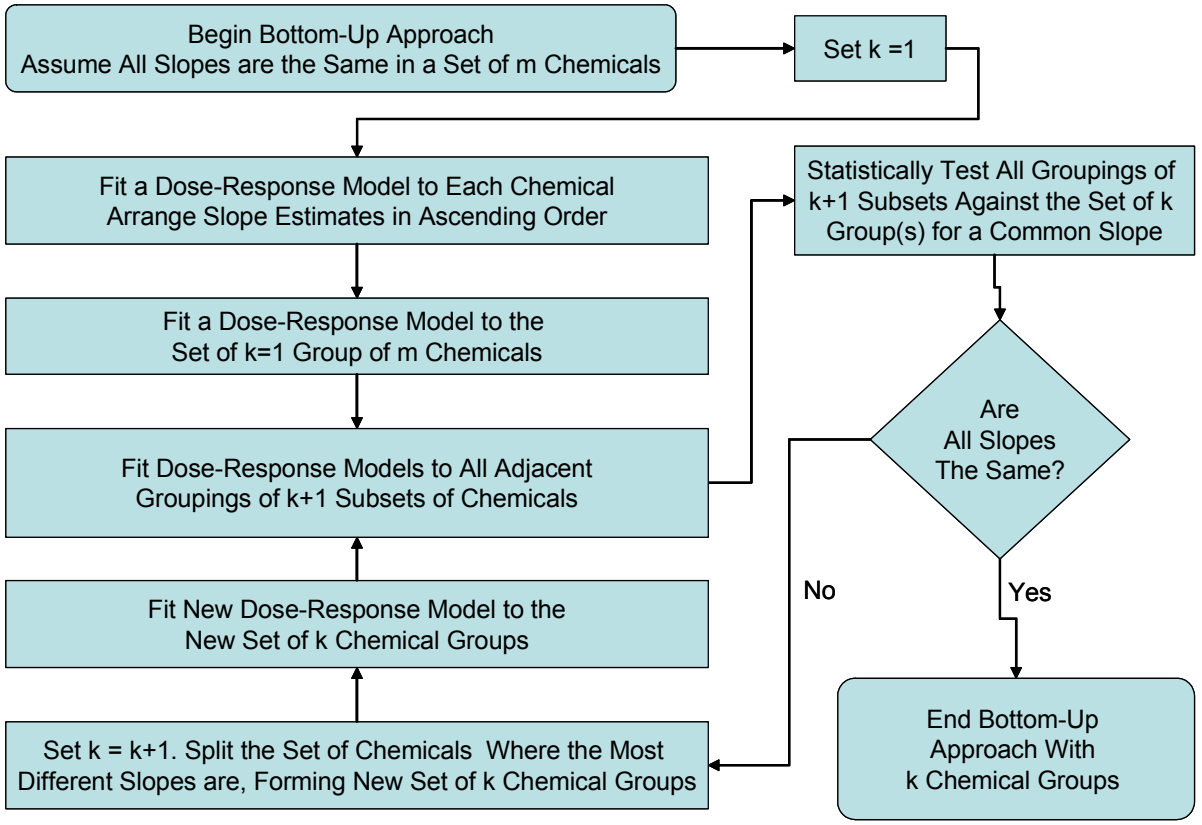

FIGURE 5-2

Flow Chart for Bottom-Up Approach

constant relative potency model M0 against the alternative model B1 of two subclasses of constant relative potency factors:

$$H_{0q}: \text{M0} \quad \text{versus} \quad H_{aq}: \text{B1}.$$

To test every possible combination of two subclasses while holding the order of the slopes constant, there are (m-1) tests. Let p_{cq} be the p-value associated with the test H_{0q} versus H_{aq}, for q = 1, ..., m-1, and let p_{cr} = Min { $p_{c1,}$ $p_{c2,\ ...}p_{cm}$} (i.e., p_{cr} is the smallest p value associated with testing for a common slope between two adjacent subclasses of chemicals). If p_{cr} is greater than a pre-specified significance level, say, α_b, then the procedure stops, and the initial model M0 where all the slopes are the same is accepted. On the other hand, if p_{cr} is less than the significance level, then the corresponding alternative model dividing the chemicals into two RPF groups, M1 = {{C1, ... C_q}, {C_{q+1}, ... , Cm}} is accepted, and the procedure continues to the next step. The algorithm repeats until a null model is accepted. Note that in the last step, if the null hypothesis is rejected, then the model that all slopes are different is concluded, {{C1}, {C2}, {C3}, {C4}, {C5}, {C6}}.

The two clustering schemes described above are tree structure classifications. The top-down algorithm forms the tree from the top. It assumes that the slopes of the chemicals are different. In each step, a chemical (or subclass of chemicals) is combined with another chemicals (or subclass of chemicals) to form a new subclass. Therefore, the number of subclasses at each step is one less than the previous step. On the other hand, the bottom-up algorithm forms a tree in a division fashion. It assumes that the slopes of the chemicals are equal. A new subclass is formed in each step. These two algorithms may result in different tree structures. In both procedures, a goodness-of-fit

test (a global test) can be performed on the terminal tree against the two trivial trees {{C1}, {C2}, {C3}, {C4}, {C5}, {C6}} and {{C1, C2, C3, C4, C5, C6}}.

5.3. CUMULATIVE RISK ASSESSMENT

The fitted dose-response model for the mixture from multiple chemical exposures can be used for quantitative risk estimation in terms of the equivalent total mixture dose of the index chemical. For a group of *m* chemicals in which the relative potency factor between any two chemicals is constant, the estimated cumulative risk from exposure to the specific doses $d_{10},...,d_{m0}$, for chemicals *1, ...*, m, respectively, is derived as,

$$F\left(d_{10},\ldots,d_{m0}\right) = F\left(\hat{\alpha}_s + \hat{\beta}\,\log\left(\sum_{t=1}^{m}\hat{\rho}_{st}d_{t0}\right)\right) \qquad (5\text{-}20)$$

where $\hat{\alpha}_s, \hat{\beta}, \hat{\rho}_{st}$ are the maximum likelihood estimates of the model parameters, and

$D = \left(\hat{\rho}_{s1}d_{10}+\ldots,\hat{\rho}_{sm}d_{m0}\right)$ is the equivalent total mixture in terms of the index chemical *s*, and $\rho_{ss} = 1$. The cumulative risk can be expressed as a response of the mixture dose in terms of the dose-response function of the index chemical,

$$P(D) = F\left(\hat{\alpha}_s + \beta\,\log d\right) \qquad (5\text{-}21)$$

Using this equation, either the effective dose (ED*p)* for a given response level *p*% or an acceptable dose level *D** corresponding to a given risk level *r* can be computed (i.e., *P*(ED*p*) = *p*% or *P*(*D**) = *r*). In general, when the relative potency factor is not constant, the estimated cumulative risk will depend on the index chemical. In this case, the average risk or the maximum risk over all possible index chemicals can be calculated.

5.3.1. Mixtures Reference Dose. The equations developed using RPFs may be useful in calculating a mixtures Reference Dose (RfD). The RfD is a "safe" level for environmental pollutants, which represents a human exposure level below which deleterious non-cancer effects are not expected to occur (U.S. EPA, 2003b). The RfD has traditionally been derived by dividing an experimental dose level, a No-Observed-Adverse-Effect Level (NOAEL) or a Lowest-Observed-Adverse-Effect Level (LOAEL) from an animal toxicity study by several uncertainty factors (UFs), and a modifying factor (MF):

$$RfD = \frac{NOAEL \text{ or } LOAEL}{UFs \ X \ MF} \tag{5-22}$$

An alternative method is to replace the NOAEL or LOAEL by a modeled benchmark dose (e.g., the lower 95% confidence limit on an ED_{10}, that is, an effective dose that produces a 10% response). These UFs are used to specifically account for uncertainty in the RfD estimate due to extrapolations across species (UF_A), within species (UF_H), across durations of exposure (UF_S), between experimental dose levels (UF_L) and from weak to strong databases (MF). In the absence of statistical treatment, the default value of these UFs has typically been set equal to 10. For a single chemical, a benchmark dose (e.g., ED_{10}) often serves as the point-of departure for low-dose extrapolation in order to minimize model dependency at low dose levels.

Using the mixture dose response models in this section, a mixtures reference dose (RfD_m) can be developed. For a mixture of components with the same mode of action, Equation 5-21 can be used to calculate the point-of-departure for the mixture.

The RfD_m in terms of an index chemical is defined as $RfD_m = EDp / UF_m$; where ED*p* is the mixture dose corresponding to a risk level of *p*% and UF_m is the uncertainty factor for the mixture. The UF_m would need to consider all of the same UFs shown above for the single chemicals RfD development. For given exposure doses, risks above the RfD_m can be calculated using an appropriate mixture dose response model (Wilkinson et al., 2000). (A complete example of the this procedure is shown in Appendix A.)

6. CONCLUSIONS

An important question in mixtures risk assessment research is how to assess a mixture containing some chemicals that share a common toxic mode of action and other chemicals that do not. Current additivity methods have evolved to handle either the former (dose addition) or the latter (response addition). Alternatively, the risk assessor may choose to do the assessment based on whole mixture data. The biostatistical methods developed in this report provide alternative methods to evaluate a mixture under three scenarios. The simple case occurs when there is certainty that a common toxic mode of action is operating, so a dose addition approach can be applied. The second case occurs when the mixtures can be divided into independent mode of action subclasses; dose addition and response addition can be integrated to make the assessment. The third case occurs when mode of action is uncertain, so a joint dose-response modeling procedure is used to create a range of risk estimates. Thus, these approaches enrich the available library of mixture risk assessment methods beyond what is currently published by the U.S. EPA (1986, 2000). Further, these approaches are available if needed for the evaluation of additional pesticide mixtures under FQPA. Finally, the results presented here are generalizable to assessments of other environmental mixtures; the risk assessments that support environmental regulations of important environmental mixtures such as dioxins, polychlorinated biphenyls, and OPs are based on concepts of additivity (U.S. EPA, 1989b, 2000, 2001b).

The research results in this report can be applied to reduce uncertainties in RPF-based risk assessments of chemical mixtures. These results also show how

mixtures risk assessments can be conducted using additivity concepts. Various sources of uncertainty exist in most mixtures risk assessments, including uncertainties addressed in this report regarding several factors:

- Common mode of action across mixture components (Sections 2, 3)
- Similarly shaped dose-response curves across mixture components (Sections 2, 5)
- Value of internal vs. external dose estimates for developing RPFs (Section 4)
- Choice of dose metric (moles vs. mass) to use in a cumulative risk assessment (Section 4)
- Cross-species extrapolation of relative potency factors (Section 4)
- Estimating risks for a mixture with two or more common mode of action subclasses (Section 5).

Biostatistical modeling in this report presents ways to combine dose-response information, partitioning the mixtures into common mode of action subclasses. These models can then be used to estimate risks for specific exposure scenarios or used to develop toxicity values, such as a reference dose for the mixture. Three RPF-based methods are discussed, reflecting what is known or uncertain about the mixture toxicology. These approaches can be applied using internal or external doses. Development of approaches based on internal doses may reduce some toxicokinetic uncertainties associated with RPFs based on administered doses. In the Chen et al. papers (2001, 2003) in Appendices A and B, external doses were used to develop statistical methods for grouping components into common mode of action subclasses. The next step in this process is to use RPFs based on internal doses and compare subclass groupings and modeling results with those developed using external doses. Recommended future RPF research on pesticide mixtures is to:

1) Develop kinetic models for pesticide mixtures in rodents.

2) Using experimental cholinesterase inhibition measures, determine RPFs based on both external and internal dose estimates for the rodent.

3) Determine if the RPFs based on internal dose estimates significantly differ from RPFs developed from external doses for the rodent.

4) Apply the biostatistical methods for grouping by common dose-response curves using RPFs based on internal and external doses and compare the groupings that result.

5) Develop kinetic models for pesticide mixtures in humans.

6) Estimate human risks using rodent cholinesterase inhibition responses, RPFs based on rodent internal doses, and human internal dose estimates using the three approaches presented in Chen et al. (2001, 2003), as appropriate.

7) Compare subclass groupings and human risk estimates for all scenarios of internal and external RPFs.

8) Evaluate the toxicity of different human exposure scenarios with the RPF models developed.

This research was undertaken to continue exploring and developing cumulative risk assessment strategies based on dose addition concepts beyond current applications and is intended to improve future applications of RPF based risk assessments.

7. REFERENCES

Chen, J.J., Y.J. Chen, G. Rice et al. 2001. Using dose addition to estimate cumulative risks from exposures to multiple chemicals. Reg. Toxicol. Pharmol. 34(1):35-41.

Chen, J.J., Y.J. Chen, L.K. Teuschler et al. 2003. Cumulative risk assessment for quantitative response data. Environmetrics. 14:339-353. (DOI:10.1002/env587)

Finney, D.J. 1971. Probit Analysis, 3rd ed. Cambridge University Press, Cambridge, England. p. 269-282.

Hewlett, P.S. and R.L. Plackett. 1959. A unified theory for quantal responses to mixtures of drugs: Non-interactive action. Biometrics. 15:591-610.

Mileson, B.E., J.E. Chambers, W.L. Chen et al. 1998. Common mechanism of toxicity: A case study of organophosphorus pesticides. Toxicol. Sci. 41(1):8-10.

NRC (National Research Council). 1988. Complex mixtures: Methods for *in vivo* toxicity testing. National Academy Press, Washington, DC.

U.S. EPA. 1980. Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA or Superfund). 42 U.S.C. s/s 9601 et seq.

U.S. EPA. 1986. Guidelines for Health Risk Assessment of Chemical Mixtures. Federal Register. 51(185):34014-34025.

U.S. EPA. 1989a. Risk Assessment Guidance for Superfund. Vol. 1. Human Health Evaluation Manual (Part A). EPA/540/1-89/002.

U.S. EPA. 1989b. Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-dioxins and -dibenzofurans (CDDs and CDFs) and 1989 Update. Risk Assessment Forum. EPA/625/3-89/016.

U.S. EPA. 1992. Guidelines for Exposure Assessment. Federal Register 57(104):22888-22938.

U.S. EPA. 1996. Safe Drinking Water Act Amendments. National Drinking Water Clearinghouse. Online. http://www.epa.gov/safewater/sdwa/text.html

U.S. EPA. 1997. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Food, Drug, and Cosmetic Act (FFDCA) As Amended by the Food Quality Protection Act (FQPA) of August 3, 1996; U.S. Environmental Protection Agency, Office of Pesticide Programs, document # 730L97001, March, 1997. Food Quality Protection Act (FQPA) Public Law 104-170. Online. http://www.epa.gov/oppfead1/fqpa/

U.S. EPA. 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. Office of Research and Development, Washington, DC. EPA/630/R-00/002. Available in PDF format at: www.epa.gov/NCEA/raf/chem_mix.htm

U.S. EPA. 2001a. National-Scale Air Toxics Assessment for 1996. Science Advisory Board Preliminary Draft. EPA-453/R-01-003 Office of Air Quality Standards and Planning, RTP, NC. Online. http://www.epa.gov/ttn/atw/nata/natsaov.html

U.S. EPA. 2001b. Preliminary Cumulative Risk Assessment for the Organophosphorus Pesticides. Office of Pesticide Programs, Washington, DC.

U.S. EPA. 2002a. Framework for Cumulative Risk Assessment. EPA/630/P-02/001A. Risk Assessment Forum, Washington, DC.

U.S. EPA. 2002b. Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity. OPP, Washington, DC. Online. http://www.epa.gov/oppfead1/trac/science/cumulative_guidance.pdf

U.S. EPA. 2002c. The Feasibility of Performing Cumulative Risk Assessments for Mixtures of Disinfection By-Products in Drinking Water. EPA/600/R-03/051 Final Draft.

U.S. EPA. 2003a. Regional/ORD Workshop on Cumulative Risk Assessment, Dallas, TX, Nov. 4-8, 2002. Washington, DC.

U.S. EPA. 2003b. Integrated Risk Information System. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Online. http://www.epa.gov/iris

Wilkinson, C.F., G.R. Christoph, E. Julien et al. 2000. Assessing the risks of exposures to multiple chemicals with a common mechanism of toxicity: How to cumulate? Reg. Toxicol. Pharmol. 31:30-43.

APPENDIX A

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USING DOSE ADDITION TO ESTIMATE
CUMULATIVE RISKS FROM EXPOSURES TO MULTIPLE CHEMICALS

Using Dose Addition to Estimate Cumulative Risks from Exposures to Multiple Chemicals

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SUMMARY

The Food Quality Protection Act (FQPA) of 1996 requires the EPA to consider the cumulative risk from exposure to multiple chemicals that have a common mechanism of toxicity. Three methods, hazard index (HI), point of departure index (PODI), and toxicity equivalence factor (TEF), have commonly been considered to estimate the cumulative risk. These methods are based on estimates of ED_{10} (point of departure) and reference doses from the dose response functions of individual chemicals. They do not incorporate the actual dose response function of the mixture from multiple chemical exposures. Dose addition is considered to be an appropriate approach to cumulative risk assessment because it assumes that the chemicals of interest act in accordance with a common mode of action (a similar action). This paper proposes a formal statistical procedure to estimate the cumulative risk by fitting the dose response model of the mixture under dose addition. The relative potency between two chemicals is estimated directly from the joint dose response model of the mixture. An example data set of four drugs representing four chemicals is used to illustrate the proposed procedure and compare it to the HI, PODI, and TEF methods.

Key Words: Chemical mixture; Low-dose extrapolation; Relative potency factor (RPF); Similar action; Toxicity equivalence factor (TEF);

1. Introduction

Regulatory agencies use risk assessment to derive acceptable levels of exposure to chemicals that may exist as contaminants in food, drinking water, air, or the environment. Risk assessment for toxic agents is usually conducted to evaluate the potential risks from exposure to a single toxic agent through a single route of exposure. Although it is important to establish safe levels of exposure for humans for each toxic agent, people frequently are exposed to many chemicals simultaneously or in sequence by different routes. The exposures to multiple chemicals could cause unexpected cumulative potential effects through various media. The risks may combine additively, multiplicatively or in some other fashion. The combined risk may be greater, or less than what would be predicted from data on individual chemicals. Concerns about the problems of multiple chemical exposure have been an important issue. The risk associated with exposure to more than one toxic chemical by different routes may be characterized by cumulative exposure and risk assessments.

Assessing the cumulative toxicological effects of multiple chemicals has been addressed from time to time (NRC, 1988; EPA, 1986, 1999a). Methods and data, which can be used to estimate the risk of exposures to multiple chemicals, have been developed over the years. But there is no consensus on appropriate statistical methods for cumulative risk assessments (CRA). The Food Quality Protection Act (FQPA) of 1996 requires that, in future risk assessments, the EPA must consider not only the risk of a single pesticide chemical residue, but also the risk of exposures to other pesticide residues and substances that have a common mechanism of toxicity. The FQPA specifically focuses on available information concerning the potential cumulative effects of such exposures.

The issue of a common mechanism of toxicity has recently been addressed by a working group of experts convened by the ILSI Risk Science Institute (RSI) (Mileson et al., 1998). The working group presented three criteria to describe a common mechanism of toxicity: 1) cause the same critical toxic effect; 2) act on the same molecular target at the same target tissue; and 3) act by

the same biochemical mechanism of action, or share a common toxic intermediate. The working group agreed that all three points are useful to apply to chemicals that may act by a common mechanism of toxicity, but did not state whether all three points must be met before a firm common mechanism of toxicity determination can be reached. It is recognized, however, that precise mechanistic information on animal or human effects of pesticide chemicals is scant. Common mechanism determinations will therefore be difficult to establish with these three points because chemicals often exhibit a different spectrum of adverse effects in different organs and tissues (Mileson et al. 1998).

Wilkinson, et al., (2000) evaluated three methods of assessment of cumulative risk from exposures to multiple chemicals: hazard index (HI), point of departure index (PODI), and toxicity equivalence factor (TEF). They also considered two the other methods of assessment: the margin of exposure (MOE) and cumulative risk index (CRI) that are the reciprocals of the PODI and HI approaches, respectively. The approach of these methods is based on estimates of reference doses or point-of-departure doses (e.g., ED_{10}) from the fitted individual dose response functions. There is no attempt to incorporate the dose response function of the mixture from combined exposures to multiple chemicals. In this paper, we propose a quantitative approach to estimating the cumulative risk by directly fitting the dose-response function of the mixture through the dose addition model.

Under the assumption of a common mode of action (chemicals are non-interactive and act on similar biological systems in eliciting a common response) for multiple chemicals, the chemicals are commonly assumed to act as if one is a simple dilution of the other. The joint action of the chemicals, then, can be described by "dose addition" (Finney, 1971). The assumption of addition of individual exposures (dose addition) to predict a cumulative toxic effect is reasonable (Wilkinson, et al., 2000). Furthermore, dose additivity is consonant with EPA policy that "pesticide chemicals that cause related pharmacological effects will be regarded, in the absence of evidence to the contrary, as having an additive deleterious actions" (CFR, 1998); also the EPA

(1986) recommended no-interaction approaches of dose addition for risk assessment of mixtures.

Let F_1 and F_2 be the dose response functions for chemical 1 and chemical 2, respectively. Under dose-addition, the response to the combination of d_1 and d_2 for chemical 1 and chemical 2, respectively, is

$$\begin{aligned} R(d_1, d_2) &= F_1(d_1 + \rho d_2) \\ &= F_2(d_1/\rho + d_2), \end{aligned}$$

where ρ is the relative potency of chemical 2 to chemical 1. When one chemical acts as if it is a simple dilution of the other, then the relative potency between the two chemicals is constant. In other words, for all response levels, the effective dose of one chemical is a constant multiple of the effective dose of the other chemical. Hewlett and Plackett (1959) viewed the concept of dose addition (similar action) in a slightly broader sense than requiring a constant relative potency between two chemicals. Mathematically, their characterization can be interpreted as allowing the relative potency factor to be different for different response levels.

Dose addition allows for summing the individual doses into an equivalent dose in terms of an index chemical, and using the index chemical's dose-response function to estimate the response from the equivalent total mixture dose. Dose addition is considered to be an appropriate approach to cumulative risk assessment because it assumes that the chemicals of interest act in accordance with a common mechanism of toxicity. The main purpose of this paper is to propose an approach to calculating cumulative risk under the broader definition of dose addition in which the relative potency is not constant (Hewlett and Plackett, 1959). The approach involves estimating the relative potencies between chemicals from the joint dose response function of the mixture through addition of the doses of individual compounds.

2. Dose Response Model for Combined Exposures

A dose response function for binary response data, denoted $P(d) = F$, relates the probability of response to the dose, d, where F is a probability distribution function. The general model can be expressed in the logarithm of dose as

$$P(d) = F(\alpha + \beta \log d), \qquad d > 0,$$

or in the un-transformed dose as

$$P(d) = F(\alpha + \beta d).$$

Two commonly used dose response models are the probit model and the logistic model. The log-probit model is

$$P(d) = c + (1 - c) \int_{-\infty}^{\alpha + \beta \log d} \frac{1}{\sqrt{2\pi}} \exp(-1/2t^2) dt$$

and the log-logistic model is

$$P(d) = c + (1 - c) \frac{\exp(\alpha + \beta \log d)}{1 + \exp(\alpha + \beta \log d)},$$

where the parameter c represents background effect and $P(d)$ is defined to be c when $d = 0$. The parameters α and β are the intercept and slope of the dose response models under $F^{-1}(P(d))$.

Consider only two chemicals and denote the dose response functions for chemical 1 and chemical 2 as

$$P_1(d) = F(\alpha_1 + \beta_1 \log d)$$

and

$$P_2(d) = F(\alpha_2 + \beta_2 \log d).$$

If the relative potency ρ of chemical 2 to chemical 1 is constant, then the dose response for one chemical can be expressed in terms of the equivalent dose of the other chemical, i.e., $P_1(d_1) = P_2(d_1/\rho) = P_1(\rho d_2) = P_2(d_2)$, where the dose d_1 of chemical 1 and d_2 of chemical 2 have an equal effect ($\rho = d_1/d_2$). Now, if $P_1(d_1) = P_2(d_1/\rho)$, then

$$\alpha_1 + \beta_1 \log d_1 = \alpha_2 + \beta_2 \log d_1/\rho.$$

The above equality holds for all d_1. In particular, letting $d_1 = 1$, the equation becomes $\alpha_1 = \alpha_2 - \beta_2 \log\rho$. This implies $\log\rho = (\alpha_2 - \alpha_1)/\beta_2$. Similarly, If $P_2(d_2) = P_1(\rho d_2)$, then

$$\alpha_2 + \beta_2 \log d_2 = \alpha_1 + \beta_1 \log d_2\rho, \text{ for all } d_2.$$

It implies analogously that $\log\rho = (\alpha_2 - \alpha_1)/\beta_1$. Hence, $\beta_1 = \beta_2$. Conversely, assume that the slopes of the dose response functions are equal ($\beta_1 = \beta_2 = \beta$). If $P_1(d_1) = P_2(d_2)$ then

$$\alpha_1 + \beta \log d_1 = \alpha_2 + \beta \log d_2.$$

The relative potency of chemical 2 to chemical 1 is $\log\rho = (\alpha_2 - \alpha_1)/\beta$. Thus, the relative potency ρ between the two chemicals is constant for all dose (response) levels. We have shown that two chemicals have a constant relative potency if and only if the slopes of the (log) dose response functions are equal.

If the dose-response functions are modeled in terms of un-transformed doses instead of log doses, then the relative potency is constant if and only if the intercepts of the dose-response functions are equal, where the relative potency is the ratio of the slopes. The remainder of this paper will address only log-dose models.

2.1 Constant Relative Potency

If two chemicals have a constant relative potency and if the joint response is dose-additive, then the dose-response function from exposure to d_1 of chemical 1 and d_2 of chemical 2 is

$$\begin{aligned} F(d_1, d_2) &= P_1(d_1 + \rho d_2) \\ &= F(\alpha_1 + \beta \log(d_1 + \rho d_2)). \end{aligned}$$

For a group of m chemicals in which the relative potency between any two chemicals is constant, the joint response of the m chemicals can be derived as

$$\begin{aligned} F(d_1, \cdots, d_m) &= P_1(d_1 + \sum_{t=2}^{m} \rho_t d_t) \\ &= F(\alpha_1 + \beta \log(d_1 + \sum_{t=2}^{m} \rho_t d_t)), \end{aligned}$$

where $\rho_t = \exp[(\alpha_t - \alpha_1)/\beta]$ is the relative potency of chemical t to the index chemical 1, $t = 2, \cdots, m$. The joint response can also be expressed in terms of any other chemical as an index chemical s,

$$F(d_1, \cdots, d_m) = F(\alpha_s + \beta \log(d_s + \sum_{t \neq s}^{m} \rho'_t d_t)),$$

where $\rho'_t = \exp[(\alpha_t - \alpha_s)/\beta]$. It can be seen that $\rho'_t = \rho_t/\rho_s$ for $t = 1, \cdot, m$, where $\rho_1 = 1$. The two models are equivalent, i.e., the estimated risk at any set of doses does not depend on the choice of index chemical.

2.2 Non-Constant Relative Potency

If the relative potency factor between chemical 1 and chemical 2 is different for different response levels, then the slopes of the dose response functions for the two chemicals are different. At the equal effective doses of d_1 for chemical 1 and d_2 for chemical 2 such that $P_1(d_1) = P_2(d_2)$, it can be shown that the equivalent dose of chemical 2 in terms of chemical 1 is

$$d_1(d_2) = \exp(\frac{\alpha_2 - \alpha_1}{\beta_1}) d_2^{\beta_2/\beta_1},$$

and the equivalent dose of chemical 1 in terms of chemical 2 is

$$d_2(d_1) = \exp(\frac{\alpha_1 - \alpha_2}{\beta_2}) d_1^{\beta_1/\beta_2}.$$

Under dose-addition, the joint response from an exposure to d_1 of chemical 1 and d_2 of chemical 2 in terms of chemical 1 is

$$\begin{aligned} F(d_1, d_2) &= P_1(d_1 + d_1(d_2)) \\ &= F(\alpha_1 + \beta_1 \log(d_1 + \rho_{12} d_2^w)), \end{aligned}$$

where $w = \beta_2/\beta_1$, and $\rho_{12} = \exp[(\alpha_2 - \alpha_1)/\beta_1]$. On the other hand, the joint response in terms of chemical 2 is

$$\begin{aligned} F(d_1, d_2) &= P_2(d_2(d_1) + d_2) \\ &= F(\alpha_2 + \beta_2 \log(d_2 + \rho_{21} d_2^{1/w}), \end{aligned}$$

where $\rho_{21} = \exp[(\alpha_1 - \alpha_2)/\beta_2]$. Note that the joint response predicted from chemical 1, $P_1(d_1 + d_1(d_2))$, will differ from that predicted from chemical 2, $P_2(d_2(d_1) + d_2))$.

For m chemicals, the combined response in terms of chemical s can be derived as

$$\begin{aligned} F(d_1, \cdots, d_m) &= P_s(d_s + \sum_{t \neq s}^{m} \rho_{st} d_t^{w_{st}}) \\ &= F(\alpha_s + \beta_s \log(d_s + \sum_{t \neq s}^{m} \rho_{st} d_t^{w_{st}})). \end{aligned}$$

The $\rho_{st} = \exp[(\alpha_t - \alpha_s)/\beta_s]$ is a potency ratio of chemical t to the index chemical s, and $w_{st} = \beta_t/\beta_s$ is the slope ratio, $t = 1, \cdots, m$, and $t \neq s$.

2.3 General Cases

For a set of m chemicals, the chemicals can be clustered into several subclasses of constant relative potency. For example, the set {{C1, C2, ..}, {Cl, C(l+1)..}, .. {Cm}} represents that the chemicals 1, 2, .. in the first subclass have constant relative potency with respect to each other as do the chemicals Cl, C(l+1) .. in the second subclass; the relative potency factor between the last chemical Cm and the other chemicals is different at different response levels. For this example, the joint response in terms of chemical 1 is

$$F(d_1, \cdots, d_m) \quad = \quad P_1((d_1 + \rho_2 d + ...) + ... + (\rho_l d_l^{w_l} + \rho_{l+1} d_{l+1}^{w_l} + ...) + ... + \rho_m d_m^{w_m}).$$

The chemicals in the same subclass will have the same slope ratio w_t $(= \beta_t/\beta_1)$. Also, the chemicals in the same subclass will have the same cumulative risk estimate, regardless of which is used as the index chemical.

3. Cumulative Risk Estimation

The fitted dose response model for the mixture from multiple chemical exposures can be used for quantitative risk estimation in terms of the equivalent total mixture dose of the index chemical. For a group of m chemicals in which the relative potency factor between any two chemicals is constant, the estimated cumulative risk from exposure to the specific doses $d_{10}, \ldots, d_{m0}$ for chemicals 1, ..., m, respectively, is

$$F(d_{10}, \cdots, d_{m0}) = F(\hat{\alpha}_s + \hat{\beta}\log(\sum_{t=1}^{m} \hat{\rho}_{st} d_{t0})),$$

where $\hat{\alpha}_s, \hat{\beta}, \hat{\rho}_{st}$ are the maximum likelihood estimates of the model parameters, and $D = \hat{\rho}_{s1} d_{10} + \cdots, \hat{\rho}_{sm} d_{m0})$ is the equivalent total mixture in terms of the index chemical s, and $\rho_{ss} = 1$. The cumulative risk can be expressed as a response of the mixture dose in terms of the dose response function of the index chemical

$$P(D) = F(\hat{\alpha}_s + \hat{\beta}\log D).$$

Using this equation, either the ED_p for a given response level $p\%$ or an acceptable dose level D^* corresponding to a given risk level r can be computed, i.e., $P(\mathrm{ED}_p) = p\%$ or $P(D^*) = r$.

In general, when the relative potency factor is not constant, the estimated cumulative risk will depend on the index chemical. In this case, the average risk or the maximum risk over all possible index chemicals can be used.

For a single chemical, a benchmark dose (e.g., ED_{10}) often serves as the so-called point-of-departure for low-dose extrapolation in order to minimize model dependency at low dose levels. The above equation can be used to calculate the point-of-departure. The reference dose for the mixture in terms of an index chemical is defined as

$$\mathrm{Ref} = \mathrm{ED}_p/\mathrm{GUF},$$

where ED_p is the mixture dose corresponding to a risk level of $p\%$ and GUP is the group uncertainty factor. For given exposure doses, the estimated risk unit with respective to the risk at the reference dose can be calculated (Wilkinson, et al. 2000).

4. An Example for Cumulative Risk Estimation

A data set of four analgesics given by Finney (1971, Chapter 6, p 104) is used as an example to illustrate the proposed procedure. These represent typical toxicological data obtained from dose response experiments. The four analgesics can be regarded as four chemicals having a common mode of toxicity. The logistic dose response function is used in the analysis,

$$P(d) = c + (1 - c)\frac{\exp(\alpha + \beta \log d)}{1 + \exp(\alpha + \beta \log d).}$$

Table 1 contains the maximum likelihood estimates with standard error estimates in parentheses and the maximum value of the log-likelihood (LL) of the fitted logistic dose response

function for the four chemicals. The ED_{10}, $ED_{.10}$, and specific exposure doses with corresponding predicted risk of the four chemicals are also listed in Table 1. The ED_{10} and $ED_{.10}$ are used later to describe the three cumulative risk assessment methods presented by Wilkinson et al. (2000). We are interested in estimating the cumulative risk at the exposure doses $d_{10} = .005, d_{20} = .010, d_{30} = .005, d_{40} = .010$. The sum of the four individual risks is 6.67×10^{-5}.

The likelihood ratio (LR) test is used to test for the equality of the slopes. The LL value under a common slope model is -729.225. The LR χ^2 statistic under the null hypothesis is 2[729.225-(209.358+157.447+139.797+221.716)] = 1.814. The χ^2 value shows no evidence of any differences among the four slopes.

The data set of the four chemicals is fitted to the model of constant relative potency given by

$$P(d_1, d_2, d_3, d_4) = c + (1 - c)\frac{\exp(\alpha_s + \beta \log(d_s + \rho_{s2}d_2 + \rho_{s3}d_3 + \rho_{s4}d_4)}{1 + \exp(\alpha_s + \beta \log(d_s + \rho_{s2}d_2 + \rho_{s3}d_3 + \rho_{s4}d_4)},$$

where ρ_{st} is the relative potency factor of chemical t to the index chemical s. Table 2 contains the maximum likelihood estimates with standard error estimates of the coefficients of the dose response function, the equivalent exposure dose D with the predicted cumulative risk, and the ED_{10} and $ED_{.10}$ using four different index chemicals ($s = 1, 2, 3, 4$). Note that ρ_a, ρ_b, and ρ_c are the estimates of the relative potency factors between chemicals relative to the index chemical. For example, when $s = 1$, then $\rho_a = \rho_{12}$, $\rho_b = \rho_{13}$, $\rho_c = \rho_{14}$. The maximum likelihood estimates of the model parameters are $\hat{c} = .056, \hat{\alpha} = -2.605, \hat{\beta} = 1.90, \hat{\rho}_{12} = 1.26, \hat{\rho}_{13} = 3.61$, and $\hat{\rho}_{14} = 0.34$. The total mixture dose is $D = .005 + 1.26 \times .010 + 3.61 \times .005 + 0.34 \times .010 = 0.0391$. The predicted cumulative risk is 1.47×10^{-4}. The predicted risk can be computed using a different index chemical. Table 2 shows that risk estimate is the same regardless of which chemical is selected as the index chemical. For a convex dose response function, the estimated (low dose) risk based on simply summing the individual risks (6.67×10^{-5} shown in Table 1) will underestimate the cumulative risk through dose addition (1.47×10^{-4} shown in Table 2) under a model of a

common mode of action.

Alternatively, using the ED_{10} as the point-of-departure, the reference dose for the mixture in terms of the index chemical 1 can be calculated by

$$\text{Ref} = 1.2820/\text{GUF}.$$

If GUF = 50, then Ref = 1.2820/50 = 0.026. This value is smaller than the mixture dose 0.039. Similarly, the reference doses for the mixture in terms of other index chemicals 2, 3, and 4 are 0.020, 0.007, and 0.076 respectively. These values are smaller than their corresponding mixture doses shown in Table 2.

For illustration purposes, assume that the relative potency factors between chemicals 1, 3, and 4 (with each other) are constant, and the relative potency factors between chemical 2 and chemicals 1, 3, and 4 are different. The four chemicals are grouped into two subclasses { {1,3,4}, {2} }. If chemical 1 is used as the index chemical (to represent the subset {1,3,4}), then the joint dose response function is

$$P(d_1, d_2, d_3, d_4) = c + (1-c)\frac{\exp(\alpha_1 + \beta_1 \log(d_1 + \rho_{12} d_2^w + \rho_{13} d_3 + \rho_{14} d_4)}{1 + \exp(\alpha_1 + \beta_1 \log(d_1 + \rho_{12} d_2^w + \rho_{13} d_3 + \rho_{14} d_4)}.$$

If chemical 2 is used as the index chemical, then dose response function becomes

$$P(d_1, d_2, d_3, d_4) = c + (1-c)\frac{\exp(\alpha_2 + \beta_2 \log(d_2 + \rho_{21} d_1^{w'} + \rho_{23} d_3^{w'} + \rho_{24} d_4^{w'})}{1 + \exp(\alpha_2 + \beta_2 \log(d_2 + \rho_{21} d_1^{w'} + \rho_{23} d_3^{w'} + \rho_{24} d_4^{w'})}.$$

Table 3 contains the maximum likelihood estimates of the model parameters. Table 3 shows that chemicals 1, 3, and 4 give the same predicted risk (1.39×10^{-4}). But the cumulative risk predicted by chemical 2 is 1.75×10^{-4}. The estimated slope ratio between the chemical 2 to chemical 1 (or 3, 4) is $w = 1.12 = 1/.89$.

5. Discussion

Wilkinson et al. (2000) described the three methods, HI, PODI, and TEF, of cumulative risk assessment based on the estimates of the ED_{10} and reference doses of individual chemicals.

For given exposure doses, the risk unit estimate can be obtained by multiplying an uncertainty factor (UF) for the chemical. In the present context, using the ED_{10} as the POD, the risk unit for the three methods is

$$\begin{aligned} \text{HI} &= \text{UF} \times (0.005/0.9110 + 0.010/1.4214 + 0.005/0.3397 + 0.010/2.9082) \\ &= \text{UF} \times 0.031 \ (= \text{PODI} = \text{TEF}), \end{aligned}$$

where UF is the common uncertainty factor for the four chemicals. The risk unit estimate is 0.31 when UF=10, and it is 3.10 when UF = 100. The $ED_{.10}$ can also be used as POD to calculate the HI, PODI, and TEF but with UF = 1, the risk unit is HI (0.005/0.0462 + 0.010/0.1855 + 0.005/0.0311 + 0.010/0.1733) = 0.380 (= PODI = TEF).

The $ED_{.10}$ can also be used as the reference dose in the TEF method as in the context of the Wilkinson et al. (2000) examples. When the ED_{10} is used as POD and $ED_{.10}$ as reference dose, applying the TEF method to estimate the risk unit will depend on the choice of the index chemicals. For example, the risk unit estimate for TEF method in terms of the index chemical 1 is

$$\frac{0.9110 \times (0.005/0.9110 + 0.010/1.4214 + 0.005/0.3397 + 0.010/2.9082)}{0.0462} = 6.05 \times 10^{-4}.$$

In the same way, the calculated risk units are 5.15×10^{-4}, 3.34×10^{-4}, and 2.35×10^{-4} for chemicals 2, 3, and 4 as the index chemical. The risk predicted from the proposed dose-addition model given in Table 2 is 1.47×10^{-4} irrespective of which chemical is selected as the index chemical.

The HI, PODI, and TEF methods all assume that the dose response functions for the chemicals considered have a similar slope. The relative potency factors among chemicals are often based on a particular effective dose ED_p (e.g., ED_{10}) of individual dose response functions. In this approach, the relative potency estimate will depend on the choice of the particular effective dose if the slopes are not estimated to be equal. For example, the relative potency between chemical 3 and chemical 1 is 0.9110/0.3397 = 2.68 based on ED_{10}, and it is 0.0462/0.0311 =

1.49 based on $ED_{.10}$ (Table 1). Because the proposed approach takes into account a common slope in fitting the joint dose response function, the estimate of the relative potency for a subset of chemicals that have the same slope is invariant to the choice of effective doses or the index chemicals. In Table 2, for example, the estimated relative potency of chemical 3 to chemical 1 is 3.61. This value can be also computed from the ratio of the ED_{10} or $ED_{.10}$ of the chemical 1 to chemical 3.

The proposed approach of fitting a single joint dose-response function to the dose response data (from all chemicals) is consistent with the current approach to a single chemical risk assessment. The fitted dose-respopnse function can be used to estimate the cumulative risk for a given set of exposure doses or to derive a reference mixture dose from a benchmark dose from the index chemical. The proposed approach is similar to the TEF method. But, unlike the TEF method, the proposed method will give the same predicted risk regardless of the choice of the index chemical under the constant relative potency model. Perhaps most importantly, the proposed approach can be used when the relative potency factor differs for different subclasses of chemicals. This flexibility, which is based on a broader than usual concept of dose addition, makes the procedure broadly applicable for estimating cumulative risk.

References

CFR (1998). Code of Federal Regulations, 40, 180.3, July 1, 1998.

EPA (1986). *Guidance for Health Risk from Exposure to Chemical Mixtures.* U.S. Environmental Protection Agency. *Fed. Reg.* **51**, 34014.

EPA (1999a). *Guidance for Conducting Health Risk Assessment of Chemical Mixtures.* U.S. Environmental Protection Agency, Washington, D.C., April 1999. Unpublished draft document.

EPA (1999b). *Proposed Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity.* U.S. Environmental Protection Agency, Washington, D.C., August 1999. Unpublished draft document.

Finney (1971). *Probit Analysis, Third Edition.* Cambridge University Press, Cambridge.

Hewlett, P. S. and Plackett, R. L. (1959). A unified theory for quantal responses to mixtures of drugs: non-interactive action. *Biometrics*, **15**, 591-610.

Mileson, B. E., Chambers, J. E., Chen, W. L., Dettbarn, W., Ehrich, M., Eldefrawi, A. T., Gaylor, D. W., Hamernik, K., Hodgson, E., Karczmar, A., Padilla, S., Pope, C., Richardson, R. J., Saunders, D. R., Sheets, L. P., Sultatos, L. G., and Wallace, K. B. (1998). Common mechanism of toxicity: A case study of organophosphorus pesticides. *Toxicol. Sci.* **41**, 8-20.

National Research Council (NRC) (1988). *Complex Mixtures: Methods for in Vivo Toxicity Testing.* Natl. Acad. Press, Washington, D. C.

Wilkinson, C. F., Christoph, G. R., Julien, E., Kelley, J. M., Kronenberg, J., McCarthy, J., and Reiss, R. (2000). Assessing the risks of exposures to multiple chemicals with a common mechanism of toxicity: How to cumulate? *Reg. Toxicol. and Pharmacol.* **31**, 30-43.

Table 1. The maximum likelihood estimates (standard errors) of the coefficients of the logistic dose-response model, and the estimated ED_{10}, $ED_{.10}$ and maximized log-likelihood value for the four chemicals.

Chemical	c	α	β	LL	ED_{10}	$ED_{.10}$	Exposure	Pred.
1	0.00	-2.05	1.58	-209.358	0.9110	0.0462	0.005	2.98×10^{-5}
	(0.27)	(1.54)	(0.71)					
2	0.12	-2.87	2.32	-157.447	1.4214	0.1855	0.010	0.11×10^{-5}
	(0.15)	(1.31)	(0.71)					
3	0.00	-0.07	1.97	-139.797	0.3397	0.0311	0.005	2.73×10^{-5}
	(0.59)	(1.41)	(0.88)					
4	0.00	-3.98	1.67	-221.716	2.9082	0.1733	0.010	0.85×10^{-5}
	(0.33)	(2.59)	(0.77)					
Sum								6.67×10^{-5}

Table 2. The maximum likelihood estimates (standard errors) of the coefficients of the joint dose response function from multiple exposures of chemicals having constant relative potency factors.

s	c	α	β	ρ_a	ρ_b	ρ_c	D	Pred.	ED_{10}	$ED_{.10}$
1	0.056	-2.605	1.90	1.26	3.61	0.34	0.0391	1.47×10^{-4}	1.2820	0.1071
	(0.09)	(0.67)	(0.30)	(0.39)	(0.03)	(0.13)				
2	0.056	-2.165	1.90	0.79	2.86	0.27	0.0310	1.47×10^{-4}	1.0170	0.0850
	(0.09)	(0.60)	(0.30)	(0.08)	(0.32)	(0.03)				
3	0.056	-0.167	1.90	0.28	0.35	0.09	0.0108	1.47×10^{-4}	0.3553	0.0297
	(0.09)	(0.32)	(0.30)	(0.03)	(0.01)	(0.04)				
4	0.056	-4.674	1.90	2.97	3.75	10.72	0.1160	1.47×10^{-4}	3.8090	0.3183
	(0.09)	(0.97)	(0.30)	(0.29)	(1.14)	(0.38)				

Table 3. The maximum likelihood estimates (standard errors) of the coefficients of the joint dose response function from multiple exposures of chemicals that do not have constant relative potency factors.

s	c	α	β	ρ_a	ρ_b	ρ_c	w	D	Pred.	ED_{10}	$ED_{.10}$
1	0.054	-2.535	1.84	1.08	3.64	0.34	1.12	0.0328	1.39×10^{-4}	1.2426	0.0958
	(0.10)	(0.67)	(0.31)	(0.41)	(0.03)	(0.28)	(0.20)				
3	0.054	-0.153	1.84	0.27	0.30	0.09	1.12	0.0090	1.39×10^{-4}	0.3405	0.0262
	(0.10)	(0.32)	(0.31)	(0.03)	(0.01)	(0.08)	(0.20)				
4	0.054	-4.541	1.84	2.97	3.21	10.80	1.12	0.0973	1.39×10^{-4}	3.6966	0.2849
	(0.10)	(0.98)	(0.31)	(0.29)	(1.18)	(0.84)	(0.20)				
2	0.054	-2.389	2.08	0.93	2.93	0.36	0.89	0.0505	1.75×10^{-4}	1.1297	0.1170
	(0.10)	(0.72)	(0.43)	(0.23)	(0.32)	(0.14)	(0.16)				

Data set of four chemicals from Finney (1971)

Chemical	Dose	Response	Total
1	1.50	19	103
1	3.00	53	120
1	6.00	83	123
2	1.50	14	60
2	3.00	54	110
2	6.00	81	100
3	0.75	31	90
3	1.50	54	80
3	3.00	80	90
4	5.00	13	60
4	7.50	27	85
4	10.00	32	60
4	15.00	55	90
4	20.00	44	60

APPENDIX B

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CUMULATIVE RISK ASSESSMENT FOR QUANTITATIVE RESPONSE DATA

Cumulative Risk Assessment for Quantitative Response Data

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SUMMARY

The Relative Potency Factor approach (RPF) is used to normalize and combine different toxic potencies among a group of chemicals selected for cumulative risk assessment. The RPF method assumes that the slopes of the dose response functions are all equal; but this method depends on the choice of the index chemical, i.e., different index chemicals will give different predicted mean estimates. This paper is part of an approach to explore and develop cumulative risk assessment strategies. As part of this approach this paper proposes a procedure for cumulative risk assessment from exposure to multiple chemicals that have a common mechanism of toxicity. We propose two classification algorithms to cluster the chemicals into subclasses such that the chemicals in the same subclass have a common slope. The joint response is estimated by fitting the dose response model of the mixture under dose addition. The proposed method will give the same predicted mean response regardless of the selection of the index chemical for the chemicals in the same subclass. The proposed method also allows one to estimate the joint response for chemicals having different slopes. An example data set of six hypothetical pesticide chemicals is used to illustrate the proposed procedure.

Key Words: Chemical mixture; Classification tree; Point of departure (POD); Relative potency factor (RPF); Similar action.

1. Introduction

Quantitative risk assessment is used to derive acceptable exposure levels or to estimate the risks from exposure to chemicals that may exist as contaminants in food, drinking water, air, or the environment. Estimation of potential risks for toxic agents is usually conducted on a single toxin by a single route of exposure. However, people frequently are exposed to many chemicals simultaneously or in sequence by different routes from different sources. Exposures to multiple chemicals could cause unexpected potential adverse effects through a variety of toxicological interactions. Various chemical components may induce similar or dissimilar effects over time. The Food Quality Protection Act (FQPA) of 1996 requires the Environmental Protection Agency (EPA) to consider not only the risk of a single pesticide chemical residue but also the risk of exposures to other pesticide residues and substances that have a common mechanism of toxicity. The FQPA specifically states the available information concerning the potential cumulative effects of such exposures. The process of risk assessment of concurrent exposure by all relevant routes for a group of compounds that cause a common toxic effect by a common mechanism is designated as cumulative risk assessment.

The issue of determining a common mechanism of toxicity has been addressed by a working group of experts convened by the International Life Sciences Institute (ILSI) Risk Science Institute (RSI) (Mileson et al., 1998). Subsequently, the EPA (EPA, 1999; http:/www.epa.gov/oppfeadl/trac/science/) has issued a guidance document for identifying pesticide chemical that have a common mechanism of toxicity. Recently, the EPA issued the results of the revised cumulative risk assessment for organophosphorus pesticides [http;//www.epa.gov/pesticides/cumulative/]. The current paper is part of an approach to continue exploring and developing cumulative risk assessment strategies. In this paper, we assume that common mechanism groups can be satisfactorily determined. In this context, a common mechanism group is defined as a group of pesticides determined to cause a common toxic effect by a common mechanism of toxicity. Such chemicals are said to occupy the same "risk cup" (EPA, 1999).

One important issue in cumulative risk assessment is how to incorporate the probability model for estimating cumulative risk. Methods and data, which can be used to conduct risk assessment of exposures to multiple chemical mixtures, have been developed over the years (NRC, 1988; EPA, 1986, 1989, 1999). Because of complexity of evaluating multiple chemicals there are no statistical methods for assessing risks from multiple chemicals that can be routinely applied to all chemical mixtures. Methods for risk assessment of chemical mixtures fall into two general approaches: 1) whole mixture of concern, and 2) component-based. The whole mixture approach involves either direct evaluation of the mixture of concern or an assessment of the mixture of concern using data available on a sufficiently similar mixture. The component-based approach considers the additive or interactive actions among the mixture components. The existing toxicological database for pesticides contains data generated primarily to evaluate the hazard potential of individual chemicals. The most widely used component-based methods are dose addition and response addition. Dose addition assumes that the chemicals act on the same biological site, similar biological systems and behave similarly in terms of the primary physiologic processes (absorption, metabolism, distribution, elimination), and elicit a common response (EPA, 2000a). Response addition assumes that the chemicals 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, a response addition model is described by statistical independence. Given that cumulative risk assessment will be based on the chemicals sharing a common toxic effect that arises by a common mechanism of toxicity, dose addition is considered to be the most appropriate model to use for estimating cumulative risk.

Dose-addition models presented in the literature are often in terms of a probability measure (e.g., Finney, 1971; EPA, 1986). Let F_1 and F_2 be the dose-response functions for chemical 1 and chemical 2, respectively. Under dose addition, the response to the combination of d_1 and d_2 for chemical 1 and chemical 2 is $F_1(d_1 + \rho d_2) = F_2(d_1/\rho + d_2)$, where ρ is the relative potency of chemical 2 to chemical 1. F_1 and F_2 are the probability of occurrence of a toxic effect for chemical 1 and chemical 2, respectively. The commonly used models are the probit, logis-

tic, and multistage models. The data for a probability model are quantal responses measured by the presence or absence of a toxic endpoint such as death. Recently, Chen et al. (2001) applied the dose-addition model approach to estimating cumulative risk for quantal effects by directly fitting the combined dose-response function for a set of chemicals in the same "risk cup".

The toxic responses from exposures to pesticide residues often are measured by a continuous quantitative value, such as altered blood concentration or altered neurological function. In the context of the FQPA, EPA has recently concluded that the organophosphorus pesticides act by a common mechanism of toxicity, which is manifested through inhibition of acetylcholinesterase (Mileson et al., 1998). The common endpoints measured in cholinesterase bioassays are plasma, red blood cell, and brain cholinesterase activity levels. In this paper, we develop a dose-addition model for quantitative response data to estimate cumulative risk.

Risk is customarily defined as the statistical probability of the occurrence of an adverse effect at a level of exposure. Dose-response models for adverse quantal response data are well defined since an adverse effect is self-evident, that is, the occurrence of an adverse effect is observed on individual subjects empirically. By contrast, a clear-cut adverse effect for continuous quantitative responses is difficult both to define and to observe unequivocally. The characterization of risk for continuous quantitative responses in terms of probability of occurrence does not naturally follow. Methods for risk estimation of continuous quantitative response data for a single toxin have been proposed by many authors (e.g., Crump, 1984; Gaylor and Slikker, 1990; Chen and Gaylor, 1992; Kodell and West, 1993; Chen et al., 1996). Dose response modeling of continuous quantitative data for cumulative risk assessment has not been developed. The main purpose of this paper is to propose an approach to estimating the cumulative response and cumulative risk of an adverse continuous quantitative effect for an individual concurrently exposed to pesticides in a common mechanism group.

2. Dose Response Model for Combined Exposure

Let $y(d)$ be a control-adjusted response variable (after a proper scaling or transformation, if necessary) of an individual exposed to a chemical at dose d. The control-adjusted response $y(d)$ is calculated either by subtracting the control mean from responses in the treatment groups (difference scale) or by dividing the responses by the control mean (ratio scale). Assume that $y(d)$ has a normal distribution with mean $E(y(d)) = \mu(d)$ and variance $\sigma.^2$ (Note that we assume a constant variance across dose groups of a chemical.) The mean response is often expressed as a linear function of the natural logarithm of dose,

$$\mu(d) = \alpha + \beta \log d,$$

where α is the response for $d = 1$. The parameters α and β are the intercept and slope of the log-dose response function, respectively.

Without loss of generality, suppose c is a critical value for an abnormally low level of response, a level below which a response is considered to be atypical. For example, c may be a certain threshold such as a 3 standard deviation reduction (difference) from the control mean or 20% reduction relative to the control mean. Under the difference scale, c can alternatively be expressed as $c = -k\sigma$, where k is appropriately chosen to yield a specific low percentage point of the distribution of unexposed individuals. For exposure to a given dose d, the proportion of the individuals with response $y(d)$ below the critical value $c = -k\sigma$ is given by

$$\begin{aligned} P(d) &= P[y(d) \leq c] \\ &= P[y(d) \leq -k\sigma] \\ &= \Phi[-k - \frac{\mu(d)}{\sigma}] \end{aligned}$$

where Φ is the standard normal cumulative distribution function. Under the ratio scale, $\mu(0) = 1$; c can be expressed as $c = 1 - k\sigma$. The probability of adverse effect at dose d becomes

$$P(d) = \Phi[-k - \frac{\mu(d) - 1}{\sigma}].$$

Note that in either case $P(0) = \Phi(-k)$. The dose d^* corresponding to the critical level $y(d^*) = c$ is regarded as a safe dose. The risk is the probability that $y(d)$ is less than or equal to the critical

value c. By expressing c in terms of k and σ the probability of an adverse effect can be calculated.

2.1 Dose Addition Model

Consider only two chemicals and denote the dose response functions for chemical 1 and chemical 2, respectively, as

$$\mu_1(d_1) = \alpha_1 + \beta_1 \log d_1$$

and

$$\mu_2(d_2) = \alpha_2 + \beta_2 \log d_2.$$

If $\mu_1(d_1) = \mu_2(d_2)$, the ratio of the equally effective doses $\rho_{12} = d_1/d_2$ is called the relative potency of chemical 2 to chemical 1. Chen et al. (2001) showed that two chemicals have a constant relative potency if and only if the slopes of the (log) dose response functions are equal, i.e., $\beta_1 = \beta_2$. The combined mean response can be derived through addition of doses of chemical 1 and chemical 2 based on the relative potency factor. Briefly, under dose addition, if two chemicals have a constant relative potency, then the dose-response function from exposure to d_1 of chemical 1 and d_2 of chemical 2 is

$$\begin{aligned} \mu(d_1, d_2) &= \mu_1(d_1 + \rho_{12}\, d_2) \\ &= \alpha_1 + \beta_1 \log(d_1 + \rho_{12}\, d_2). \end{aligned}$$

If the relative potency factor between chemical 1 and chemical 2 is different for different response levels, the joint response from exposure to d_1 of chemical 1 and d_2 of chemical 2 in terms of chemical 1 is

$$\mu(d_1, d_2) = \alpha_1 + \beta_1 \log(d_1 + \rho_{12}\, d_2^{w_{12}}),$$

where $w_{12} = \beta_2/\beta_1$, and $\rho_{12} = \exp[(\alpha_2 - \alpha_1)/\beta_1]$. The cumulative response from exposure to chemical 1 and chemical 2 can also be expressed in terms of chemical 2. However, if the relative potency is not constant, then the response predicted based on chemical 1 will differ from that

predicted based on chemical 2.

For a set of m chemicals, the chemicals can be clustered into several subclasses of constant relative potency $\{S_1, S_2, ..., S_k\}$, where each S_i consists of chemicals having constant relative potency. For example, the set $\{\{c_1, c_2, ..\}, \{c_q, c_{q+1}..\}, ..\{c_m\}\}$ represents that the chemicals $c_1, c_2, ..$ in the first subclass have constant relative potency with respect to each other as do the chemicals $c_q, c_{q+1}, ..$ in the second subclass; the relative potency factor between the last chemical c_m and the other chemicals is different at different response levels. For notation simplification, let $c_i = i$. The joint dose response function from exposure to the set of m chemicals $\{\{1, 2, ..\}, \{q, q+1..\}, ..\{m\}\}$ in terms of chemical 1 (called the index chemical) is

$$\mu(d_1, \cdots, d_m) \;=\; \mu_1[(d_1 + \rho_{12}d_2 + ...) + ... + (\rho_{1q}d_q^{w_{1q}} + \rho_{1(q+1)}d_{q+1}^{w_{1q}} + ...) + ... + \rho_{1m}d_m^{w_{1m}}].$$

The chemicals in the same subclass will have the same slope ratio w_{1t} $(= \beta_t/\beta_1)$, and give the same predictive estimate, regardless of which is used as the index chemical.

One difficulty with the use of the above approach is that the estimated combined response will depend on the subclass in which the index chemical is selected, a different subclass will predict a different estimate. An alternative approach is to estimate the dose-response function for the chemicals within each subclass, and calculate the joint dose response function as the sum of the dose response functions of the subclasses. For the example above, the joint dose-response function is

$$\mu(d_1, \cdots, d_m) \;=\; \mu_1(d_1 + \rho_{12}d_2 + ...) + ... + \mu_q(d_q + \rho_{q(q+1)}d_{q+1} + ...) + ... + \mu_m(d_m).$$

2.2 Maximum Likelihood Estimation

Let y_{ijl} denote the control-adjusted response data for the j-th observation at the dose level d_{il} from the l-th chemical ($j = 1, \cdots, n_{il}, i = 1, \cdots, g_l$, and $l = 1, \cdots, m$), where n_{il} denotes the

number of subjects in dose group i from chemical l, and g_l is the number of dose groups from chemical l. Suppose y_{ijl} is normally distributed with mean $\mu_l(d_{il})$ and variance σ_l^2. Estimation of the mean and variance parameters for an individual chemical can be obtained by the maximum likelihood method. The log-likelihood function for the chemical l is

$$LL = -\frac{1}{2}\sum_{i=1}^{g_l}\sum_{j=1}^{n_{il}}\left(\frac{[y_{ijl}-\mu_l(d_{il})]^2}{\sigma_l^2} + \log 2\pi\sigma_l^2\right),$$

where $\mu_l(d_{il}) = \alpha_l + \beta_l \log d_{il}$. The log-likelihood function for the m chemicals in terms of the chemical s (the index chemical) is

$$LL = -\frac{1}{2}\sum_{l=1}^{m}\sum_{i=1}^{g_l}\sum_{j=1}^{n_{il}}\left(\frac{[y_{ijl}-\mu_s(D_{il})]^2}{\sigma_l^2} + \log 2\pi\sigma_l^2\right),$$

where $\mu_s(D_{il}) = \alpha_s + \beta_s \log D_{il}$ and $D_{il} = d_{is} + \sum_{t\neq s}^{m} \rho_{st} d_{it}^{w_{st}}$.

Denote the maximum likelihood estimate (MLE) of α_s, β_s, ρ_{st}, w_{st}, and σ_l^2 as $\hat{\alpha_s}$, $\hat{\beta}_s$, $\hat{\rho_{st}}$, $\hat{w_{st}}$, and $\hat{\sigma_l^2}$, respectively. If the control-adjusted response y_{ijl} is measured on the difference scale, then the estimated cumulative risk from exposure to the m chemicals in terms of the chemical s can be derived from

$$\hat{P(D)} = \Phi\left[-k - \frac{\hat{\mu}_s(D)}{\hat{\sigma}_s}\right].$$

If y_{ijl} is measured on the ratio scale, then the risk estimate is given by

$$\hat{P(D)} = \Phi\left[-k - \frac{[\hat{\mu}_s(D)-1]}{\hat{\sigma}_s}\right].$$

In both cases, the estimated probability $\hat{P(D)}$ will depend on the standard deviation of a selected index chemical.

3. Tree Classification Algorithms

In this section, we propose two classification algorithms to cluster a group of chemicals into subclasses of constant relative potency factors. Since two chemicals have a constant relative

potency if and only if the slopes of the (log) dose response functions are equal, the clustering algorithm is based on testing for the equality of the slopes of dose response functions. Either the likelihood ratio test (LR) or the analysis of variance F test can be used for the comparison.

The procedure begins with a fitting of each individual dose response function for the m chemicals. Let $\hat{\beta}_1, \hat{\beta}_2, \cdots, \hat{\beta}_m$ denote the MLEs of the slopes of the dose response functions. The estimates of the m slopes can be arranged in an ascending order:

$$\hat{\beta}_{c_1} \leq \hat{\beta}_{c_2} \leq \cdots \leq \hat{\beta}_{c_m}.$$

That is, the chemical c_1 has the smallest slope estimate, the chemical c_2 has the second smallest slope estimate, and so on. Two tree classification algorithms, top-down and bottom-up, are proposed.

In the top-down classification, the procedure starts with the model that the slopes of the m chemicals are all different, denoted as $\mathbf{M0} = \{\{1\}, \{2\}, \cdots, \{m\}\}$. Consider the hypothesis of comparing two adjacent slopes,

$$\mathrm{H}_{0q} : \beta_{c_q} = \beta_{c_{q+1}} \quad \text{versus} \quad \mathrm{H}_{aq} : \beta_{c_q} \neq \beta_{c_{q+1}}$$

for $q = 1, 2, \cdots, m-1$. Under the null hypothesis H_{0q}, the dose addition model for the mixture of chemical c_q and chemical c_{q+1} is

$$\mu(d_q, d_{q+1}) \quad = \quad \alpha'_{c_q} + \beta'_{c_q} \log(d_q + \rho_{c_q c_{q+1}}\, d_{q+1}).$$

The null model H_{0q} can be represented by the partition set $\mathbf{B1}$= $\{\{c_1\}$, ...$\{c_q, c_{q+1}\}$, ...$\{c_m\}\}$. The hypothesis of comparing two adjacent slopes, equivalently, can be expressed in terms of testing the two models:

$$\mathrm{H}_{0q} : \mathbf{B1} = \{\{c_1\},\ ...,\ \{c_q, c_{q+1}\}, ...,\ \{c_m\}\} \quad \text{versus} \quad \mathrm{H}_{aq} : \mathbf{M0} = \{\{c_1\}, \{c_2\}, \cdots, \{c_m\}\}.$$

Let p_{c_q} be the p-value associated with the test H_{0q} versus H_{aq}, for $q = 1, 2, \cdots, m-1$; and let p_{c_r} = Max $\{p_{c_1}, p_{c_2}, \cdots, p_{c_{m-1}}\}$. If p_{c_r} is less than a pre-specified significance level, say, α_t, then the

procedure stops, and the model **M0** that the slopes of the m chemicals are different is concluded. On the other hand, if p_{c_r} is greater than the significance level, then the chemicals c_r and c_{r+1} can be classified into the one subclass. That is, the model **M1**= $\{\{c_1\}, ...,\{\ c_r, c_{r+1}\ \}, ...\}$ is accepted, and the procedure continues to the next step.

Under the model **M1**, the two chemicals c_r and c_{r+1} can be treated as one chemical. Let $\hat{\beta}'_{c_r}$ be the MLE of the common slope for the two chemicals c_r and c_{r+1}. The $(m-1)$ slope estimates listed in the ascending order become

$$\hat{\beta}_{c_1} \leq \cdots \hat{\beta}_{c_{r-1}} \leq \hat{\beta}'_{c_r} \leq \hat{\beta}_{c_{r+2}} \cdots \leq \hat{\beta}_{c_m}.$$

That is, the two individual slope estimates $\hat{\beta}_{c_r}$ and $\hat{\beta}_{c_{r+1}}$ are replaced by their common slope estimate $\hat{\beta}'_{c_r}$. The same algorithm is applied by performing $m-2$ comparisons of two adjacent estimates. The hypothesis can be expressed as

$$\mathrm{H}_{0q} : \mathbf{B2} \quad \text{versus} \quad \mathrm{H}_{aq} : \mathbf{M1}$$

where

$$\begin{aligned}
\mathbf{B2} &= \{...,\{c_q, c_{q+1}\},...,\{c_r, c_{r+1}\}, ...\}, && \text{if } q \neq (r-1) \text{ or } q \neq (r+1) \\
&= \{...,\{c_{r-1}, c_r, c_{r+1}\},...\}, && \text{if } q = (r-1) \\
&= \{...,\{c_r, c_{r+1}, c_{r+2}\}, ...\}, && \text{if } q = (r+1).
\end{aligned}$$

Again, if the largest p-value is less than the significance level, then the procedure stops, and the model **M1** is concluded. If the largest p-value is greater than the significance level, then the null model is accepted and the procedure continues to the next step. Note that in the last step, if the null hypothesis is not rejected, then the model $\{\{\ c_1, c_2, \cdots, c_m\}\}$ that all slopes are equal is accepted.

In the bottom-up classification, the procedure starts with the model that the slopes of the m chemicals are equal, denoted as **M0** $= \{\{c_1, c_2, \cdots, c_m\}\}$. (We use the same notations to illustrate the parallelism between the two classification schemes.) Consider the model **B1** =

$\{\{c_1, ...c_q\}, \{c_{q+1}, \cdots, c_m\}\}$ constructed by the split of **M0** into two subclasses, $q = 1, 2, \cdots, m-1$. Consider the hypothesis of a constant relative potency model **M0** against the alternative model **B1** of two subclasses of constant relative potency factors:

$$\mathrm{H}_{0q} : \mathbf{M0} \quad \text{versus} \quad \mathrm{H}_{aq} : \mathbf{B1}.$$

There are (m-1) tests. Let p_{c_q} be the p-value associated with the test H_{0q} versus H_{aq}, for $q = 1, \cdots, m-1$, and let $p_{c_r} = \text{Min } \{p_{c_1}, p_{c_2}, \cdots, p_{c_{m-1}}\}$. If p_{c_r} is greater than a pre-specified significance level, say, α_b, then the procedure stops and the model **M0** is accepted. On the other hand, if p_{c_r} is less than the significance level, then the corresponding alternative model **M1**= $\{\{c_1...c_r\}, \{c_{r+1}, \cdots, c_m\}\}$ is accepted, and the procedure continues to the next step. The algorithm repeats until a null model is accepted. Note that in the last step, if the null hypothesis is rejected, then the model that all slopes are different is concluded, $\{\{c_1\}, \{c_2\}, \cdots, \{c_m\}\}$.

The two clustering schemes described above are tree structure classifications. The top-down algorithm forms the tree from the top. It assumes that the slopes of the chemicals are different. In each step, a chemical (or subclass of chemicals) is combined with another chemicals (or subclass of chemicals) to form a new subclass. Therefore, the number of subclasses at each step is one less than the previous step. On the other hand, the bottom-up algorithm forms a tree in a division fashion. It assumes that the slopes of the chemicals are equal. A new subclass is formed in each step. These two algorithms may result in different tree structures. In both procedures, a goodness-of-fit test (a global test) can be performed on the terminal tree against the two trivial trees {1,2,...,m} and {{1,2,...,m}}.

4. An Example

A data set consisting of a group of six chemicals was constructed for the example. The data are the measures of different cholinesterase activity levels. These data represent typical endpoints measured in a cholinesterase bioassay. Table 1 shows the sample size (n), mean response, and

standard error (S.E.) for each of the five dose groups of the six chemicals. The control means for the six chemicals are 340, 345, 334, 304, 359, and 450. The Bartlett test indicates that a constant variance model among dose groups is rejected for every chemical. Therefore, a natural logarithmic transformation of the response is applied to achieve a constant variance. The constant variance model for a given chemical appears to be adequate for the transformed data (Bartlett test). The transformed data are then adjusted by subtracting their respective control means. The linear dose-response function using the natural logarithm of the dose,

$$\mu(d) = \alpha + \beta \log(d).$$

is fit to the control-adjusted data for each chemical. Table 2 contains the maximum likelihood estimates (MLEs) with standard error estimates in parentheses and the log-likelihood (LL) of the fitted dose response function for each of the six chemicals. The six slope estimates listed in ascending order are given as

$$\hat{\beta}_3(-0.289) < \hat{\beta}_4(-0.260) < \hat{\beta}_5(-0.232) < \hat{\beta}_2(-0.221) < \hat{\beta}_1(-0.212) < \hat{\beta}_6(-0.169).$$

The likelihood ratio test is used in the analysis. The significance level for the top-down approach is set to be α_t=0.25, and for the bottom-up approach is $\alpha_b = 0.05$.

In the first step of the top-down classification the different relative potency model **M0**= {{3},{4},{5},{2},{1},{6}} is compared with each of the five models: $\mathbf{B1}_1$ ={{3,4},{5},{2},{1},{6}}, $\mathbf{B1}_2$={{3},{4,5},{2},{1},{6}}, $\mathbf{B1}_3$={{3},{4},{5,2},{1},{6}}, $\mathbf{B1}_4$={{3},{4},{5},{2,1},{6}}, and $\mathbf{B1}_5$={{3},{4},{5},{2},{1,6}}. The model $\mathbf{B1}_3$ gives the largest p-value 0.6048 (> 0.25). Therefore, the model $\mathbf{B1}_3$ is used as the null model in the next step, and the procedure continues. Table 3 provides the details of the analysis in each step. This procedure concludes that the six chemicals are classified into three subclasses as {{3,4},{5,2,1},{6}}.

In the first step of the bottom-up classification, the constant relative potency model **M0** = {{3,4,5,2,1,6}} is compared with each of the five models: $\mathbf{B1}_1$ ={{3},{4,5,2,1,6}}, $\mathbf{B1}_2$={{3,4},{5, 2,1,6}}, $\mathbf{B1}_3$={{3,4,5},{2,1,6}}, $\mathbf{B1}_4$={{3,4,5,2},{1,6}}, $\mathbf{B1}_5$={{3,4,5,2,1},{6}}. The details of

analysis in given in Table 4. The bottom-up classification comes to the same three subclasses of constant relative potency model, {{3,4},{5,2,1},{6}}. In this example, the top-down algorithm requires four steps, while the bottom-up algorithm requires three steps.

The goodness-of-fit test can be performed using the likelihood ratio test to compare the terminal tree {{3,4},{5, 2,1},{6}} against the trivial trees {{3},{4},{5},{2},{1},{6}} and {{3,4,5,2,1,6}} for the top-town and bottom-up procedures, respectively. In the top-down procedure, the p-value associated with the test of comparing the null model {{3,4},{5,2,1},6} and the alternative model {{3},{4},{5},{2},{1},{6}} is greater than the significance level 0.05 (p-value=0.4984). Therefore, we conclude that the model with the three subclasses of constant relative potency {{3,4},{5,2,1},{6}} is adequate. Similarly, the goodness-of-fit test indicates a significant fit of the same model with the p-value 0.00246 (< 0.05) by testing the null mode1 {{3,4,5,2,1,6}} against the alternative model {{3,4},{5,2,1},{6}} for the bottom-up classification.

Both classification algorithms indicate that the six chemicals can be grouped into the three subclasses {3,4},{5,2,1},{6} of constant relative potency factors. The data set of six chemicals can be fitted based on the three subclasses. For example, if chemical 1 is used as the index chemical (to represent the subset {5,2,1}), then the joint dose-response function is

$$\mu(d_1, \cdots, d_6) \;=\; \alpha_1 + \beta_1 \log(d_1 + \rho_{12} d_2 + \rho_{15} d_5 + \rho_{13} d_3^{w_{13}} + \rho_{14} d_4^{w_{13}} + \rho_{16} d_6^{w_{16}}).$$

Using chemical 2 or chemical 5 as an index chemical, it will have a similar dose response function and give the same prediction at given exposure levels. If chemical 3 is chosen as the index chemical (to represent the subclass {3,4}), the joint dose-response function becomes

$$\mu(d_1, \cdots, d_6) \;=\; \alpha_3 + \beta_3 \log(d_3 + \rho_{34} d_4 + \rho_{31} d_1^{w_{31}} + \rho_{32} d_2^{w_{31}} + \rho_{35} d_5^{w_{31}} + \rho_{36} d_6^{w_{36}}).$$

Finally if chemical 6 is used to be the index chemical, then the joint dose-response function is given by

$$\mu(d_1, \cdots, d_6) \;=\; \alpha_6 + \beta_6 \log(d_6 + \rho_{61} d_1^{w_{61}} + \rho_{62} d_2^{w_{61}} + \rho_{65} d_5^{w_{61}} + \rho_{63} d_3^{w_{63}} + \rho_{64} d_4^{w_{63}}).$$

Table 5 (columns 1-11) contains the maximum likelihood estimates with standard errors of the model parameters for the different index chemicals. The notations ρ_a, ρ_b, ρ_c, ρ_d, ρ_e, w_a, and w_b denote the relative potency factors and slope ratios between chemicals relative to the index chemical, and σ is the standard deviation of the index chemical. Suppose we are interested in a cumulative risk assessment at the exposure doses $d_1 = 0.030$, $d_2 = 0.035$, $d_3 = 0.020$, $d_4 = 0.020$, $d_5 = 0.030$, and $d_6 = 0.002$. The equivalent exposure dose D can be estimated using an index chemical. For instance, when the index chemical $s = 1$, the maximum likelihood estimates of the coefficients are $\hat{\rho_{12}} = 1.032$, $\hat{\rho_{15}} = 1.600$, $\hat{\rho_{13}} = 0.437$, $\hat{\rho_{14}} = 0.273$, $\hat{\rho_{16}} = 4.718$, $\hat{w_{13}} = 1.215$, $\hat{w_{16}} = 0.762$, and $\hat{\sigma_1} = 0.171$. The total mixture dose is $D = 0.030 + 1.032 \times 0.035 + 1.600 \times 0.020 + 0.437 \times (0.020)^{1.215} + 0.273 \times (0.030)^{1.215} + 4.718 \times (0.002)^{0.762} = 0.2560$. The predicted mean response is -0.4585 or, taking anti-logarithm, a 36.8% reduction of activity of cholinesterase. The total mixture dose and predicted responses are shown in the last two columns of Table 5. It can be seen that the chemicals 1, 2,and 5 give the same predicted mean response of -0.4585 as do the chemicals 3 and 4 (-0.5247).

Alternatively, the combined response may be computed for each subclasses of chemicals with the joint dose-response function being the sum of the three dose-response functions for the three subclasses

$$\mu(d_1, \cdots, d_6) \quad = \quad [\alpha_1 + \beta_1 \log(d_1 + \rho_{12} d_2 + \rho_{15} d_5)] + [\alpha_3 + \beta_3 \log(d_3 + \rho_{34} d_4)] + [\alpha_6 + \beta_6 \log d_6)].$$

The mixture dose for the subclass {1,2,5} is $D = 0.030 + 1.032 \times 0.035 + 1.600 \times 0.020 = 0.1141$ with chemical 1 as index chemical. The predictive response is -0.2796. Similarly, the mixture dose for the subclass {3,4} is $D = 0.3360$ (chemical 3 as index chemical) with the predictive response -0.2844, and the predictive response for chemical 6 is -0.0548. The estimated cumulative response becomes (-0.2796)+(-0.2833)+(-0.0548)=-0.6177. This alternative approach uses a response addition to combine results from the dose-additive subclasses.

The estimate of adverse probability based on the joint dose-response function from exposure to the six chemicals can be calculated in terms of the critical value $c = -k\sigma$. For $k = 3$, the cumulative risk is the probability of the control-adjusted response less than or equal to three standard-deviation units. For chemical 1, 3 standard deviations below the control mean is 0.513; this value corresponds to exp(-0.513)=0.600 in terms of original measurement. Therefore, the critical value can be interpreted as 40% reduction from the control mean. The estimated probability is computed based on the choice of the index chemical. If the index chemical $s = 1$ and $k = 3$, then the cumulative risk for six chemicals in terms of chemical 1 is

$$P(D) = \Phi\left[-3 - \frac{\hat{\mu}_1(D)}{\hat{\sigma}_1}\right] = \Phi\left[-3 - \frac{-0.4585}{0.171}\right] = 0.3748.$$

The cumulative risk estimate can be interpreted as follows. Assume that a 40% (or more) reduction of a cholinesterase level, as compared to the mean of unexposed individuals, is considered to be abnormal, then the probability of an adverse effect for an individual exposed to the doses $d_1 = 0.030$, $d_2 = 0.035$, $d_3 = 0.020$, $d_4 = 0.020$, $d_5 = 0.030$, and $d_6 = 0.002$ of the six chemicals is 0.3748. For the alternative approach, the cumulative risk (in terms of chemical 1) is

$$P^a(D) = \Phi\left[-3 - \frac{-0.6177}{0.171}\right] = 0.7368.$$

The estimated cumulative risk of the joint response depends on the standard deviation of the index chemical. Therefore, different index chemicals (even in the same subclass) will give different risk estimates. Table 6 contains the predicted risks from the individual dose-response functions at the given exposure level (column 2), and the cumulative risk predicted by six different index chemicals (columns 3-4).

5. Disscussion

EPA (2000b) recommended using the Relative Potency Factor (RPF) approach to normalize and combine the different toxic potencies among the chemicals for cumulative risk assessment. An initial step of the RPF approach is to identify a point of departure (POD). A POD is

generally defined as a point estimate of the dose or exposure level that is used to depart from the observed range of empirical response (or incidence) data for the purpose of extrapolation (EPA, 2000b). In the case of a cumulative risk assessment, POD is a dose reflecting a uniform response for the common toxic effect for each chemical. The RPF is defined as the ratio of the POD of the index chemical to that of each other chemical in the group. The exposure dose to each chemical is multiplied by the RPF to express all exposures in terms of the index chemical. The summation of these values provides a total combined exposure dose expressed in terms of the index chemical for prediction. In the present context, suppose that the estimated dose corresponding to the predicted mean response of -0.05 is defined to be the POD, such that $\hat{\alpha} + \hat{\beta}\log(\text{POD}) = -0.05$. For the given exposure doses considered in this example, Table 7 contains the estimate of POD, the total combined (equivalent) exposure dose, predicted response, and the cumulative risk estimate for each index chemical. For example, the total exposure normalized to the chemical 1 is

$$\begin{aligned} \mathrm{d}_{RPF} &= 0.030(0.0327/0.0327) + 0.035(0.0327/0.0387) + 0.020(0.0327/0.1782) \\ &+ 0.020(0.0327/0.1894) + 0.030(0.0327/0.0315) + 0.002(0.0327/0.0019) = 0.1965. \end{aligned}$$

The predicted mean response associated with the total combined exposure d_{RPF} can be estimated by using the individual dose response model of the index chemical 1

$$\begin{aligned} \text{Pred}(\mathrm{d}_{RPF}) &= \hat{\alpha_1} + \hat{\beta}_1 \log(\mathrm{d}_{RPF}) \\ &= -0.774 + (-0.212) \times \log(0.1965) = -0.4290. \end{aligned}$$

The cumulative risk estimates using the RPF method is calculated as

$$\mathrm{P}(\mathrm{d}_{RPF}) = \Phi\left[-3 - \frac{-0.4290}{0.171}\right] = 0.3169.$$

Tables 6 and 7 show that the risk estimates obtained from the RPF method are, on average, slightly smaller than those obtained from the proposed model. On the other hand, the estimated risk based on simply summing the individual probabilities (0.0155, second column of Table 6) will heavily understate the risk estimated either from RPF method or from the proposed method,

as does the predicted mean response (-0.3001, shown in Table 2). The alternative version of the proposed approach, which combines dose addition and response addition, gives substantially higher risk estimates than the version that employs dose addition only.

The proposed dose addition model is similar to the RPF method. Although the RPF method assumes that the dose response functions for all chemicals have a similar slope (a constant relative potency), different index chemicals will give different predicted mean estimates. The proposed method does incorporate the actual dose response function of the mixture from multiple chemical exposures. The method allows one to estimate the joint response for the chemicals in a common mechanism group but having different relative potency factors. It will give the same predicted mean response regardless of the selection of the index chemical for the chemicals in the same subclass, but risk estimates will depend on the variance of the index chemical.

References

CFR (1998). Code of Federal Regulations, 40, 180.3, July 1, 1998.

Chen, J. J., Chen, Y-J, Rice, G., Teuschler, L. K, Hamernik, K., Protzel, A., and Kodell, R. L. (2001). Using Dose Addition to Estimate Cumulative Risks from Exposures to Multiple Chemicals. *Reg. Toxicol. and Pharmacol.*, **34**, 35-41.

Chen, J. J. and Gaylor, D. W. (1992). Dose response modeling of quantitative response data for risk assessment. *Commun. Stat. Theory Methods*, **21**, 2367-2381.

Chen, J. J., Kodell, R. L., and Gaylor, D. W. (1996). Risk assessment for nonquantal toxic effects. *Toxicology and Risk Assessment*, 503-513.

Crump, K. S. (1984). A new method for determining allowable daily intake. *Fundam. Appl. Toxicol.*, **4**, 854-871.

Environmental Protection Agency (1986). *Guidance for Health Risk from Exposure to Chemical Mixtures. Fed. Reg.*, **51**, 34014.

Environmental Protection Agency (1999). *Guidance for identifying Pesticide Chemicals and Other Substances that have a Common Mechanism of Toxicity. Fed. Reg.* **64**, 5795-5796.

Environmental Protection Agency (2000a). *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures.* U.S. Environmental Protection Agency, Risk Assessment Forum. EPA/630/R-00/002.

Environmental Protection Agency (2000b). *Proposed Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity.* U.S. Environmental Protection Agency, Washington, D.C., June 2000. Unpublished draft document.

Finney (1971). *Probit Analysis, Third Edition.* Cambridge University Press, Cambridge.

Gaylor, D. W. and W. L. Slikker (1990). Risk assessment for neurotoxic effects. *Neurotoxicology*, **11**, 211-218.

Hewlett, P. S. and Plackett, R. L. (1959). A unified theory for quantal responses to mixtures of drugs: non-interactive action. *Biometrics*, **15**, 591-610.

Kodell, R. L. and R. W. West (1993). Upper confidence limits on excess risk for quantitative responses. *Risk Anal.*, **13**, 177-182.

Mileson, B. E., Chambers, J. E., Chen, W. L., Dettbarn, W., Ehrich, M., Eldefrawi, A. T., Gaylor, D. W., Hamernik, K., Hodgson, E., Karczmar, A., Padilla, S., Pope, C., Richardson, R. J., Saunders, D. R., Sheets, L. P., Sultatos, L. G., and Wallace, K. B. (1998). Common mechanism of toxicity: A case study of organophosphorus pesticides. *Toxicol. Sci.*, **41**, 8-20.

National Research Council (NRC) (1988). *Complex Mixtures: Methods for in Vivo Toxicity Testing.* Natl. Acad. Press, Washington, D. C.

Wilkinson, C. F., Christoph, G. R., Julien, E., Kelley, J. M., Kronenberg, J., McCarthy, J., and Reiss, R. (2000). Assessing the risks of exposures to multiple chemicals with a common mechanism of toxicity: How to cumulate? *Reg. Toxicol. and Pharmacol.*, **31**, 30-43.

Table 1: A hypothetical group of six chemicals*

Chemical	Dose	n	Mean†	S.E.	Chemical	Dose	n	Mean	S.E.
1	control	5	340.0	6.97	4	control	10	304.0	5.65
	0.02	5	323.8	15.19		0.03	10	382.3	4.93
	2.3	5	167.0	11.63		1.1	10	266.4	6.27
	22.5	5	80.0	3.41		15.0	10	87.6	1.87
	213	5	45.6	1.63		168	10	44.4	1.51
2	control	5	345.0	15.20	5	control	5	359.0	9.75
	0.017	5	360.8	26.58		0.019	5	299.8	18.57
	1.7	5	187.4	12.92		1.3	5	220.0	13.49
	17.0	5	74.4	1.50		13.8	5	92.6	1.86
	177	5	50.2	3.80		189	5	35.0	2.55
3	control	5	334.0	7.52	6	control	5	450.0	13.03
	0.05	5	360.8	8.05		0.01	5	301.6	24.24
	2.0	5	268.8	8.71		0.1	5	264.2	21.23
	19.0	5	68.6	1.99		10.8	5	104.6	6.09
	205	5	38.0	2.77		250	5	59.6	8.89

∗ Data represent hypothetical events for inhibition of the activity of the enzyme of cholinesterase in laboratory animals treated with increasing doses of six different chemicals.

† Mean activity of cholinesterase after dosing.

Table 2: The Maximum Likelihood Estimates (Standard Errors) of the coefficients of the individual dose response model, and Log-likelihood values for the six chemicals

Chemical	α	β	σ	LL	d	Pred(d)*
1	-0.774	-0.212	0.170	7.0439	0.030	-0.0316
	(0.041)	(0.011)	(0.027)			
2	-0.768	-0.221	0.201	3.6799	0.035	-0.0278
	(0.047)	(0.013)	(0.032)			
3	-0.548	-0.289	0.322	-5.6999	0.020	-0.0832
	(0.080)	(0.023)	(0.051)			
4	-0.483	-0.260	0.233	1.5159	0.020	-0.0642
	(0.039)	(0.012)	(0.026)			
5	-0.853	-0.232	0.307	-4.7730	0.030	-0.0385
	(0.072)	(0.020)	(0.049)			
6	-1.104	-0.169	0.248	-0.4775	0.002	-0.0548
	(0.056)	(0.014)	(0.039)			
sum				1.2891	0.1202	-0.3001

$*$ Pred(d) is the natural logarithm of the predicted response.

Table 3: The MLEs of coefficients and LL values from the fitted dose response functions for the Steps in the top-down procedure

Step	Order of slopes	Null model	Alternative model	$\hat{\beta}$	LL	p-value
1	$\beta_3 < \beta_4 < \beta_5 < \beta_2 < \beta_1 < \beta_6$	{{3,4},{5},{2},{1},{6}}	{{3},{4},{5},{2},{1},{6}}	-0.269	0.6422	0.2554
		{{3},{4,5},{2},{1},{6}}		-0.250	0.5259	0.2167
		{{3},{4},{5,2},{1},{6}}		**-0.227**	**1.1552**	**0.6048**
		{{3},{4},{5},{2,1},{6}}		-0.216	1.1416	0.5870
		{{3},{4},{5},{2},{1,6}}		-0.187	-1.9911	0.0104
2	$\beta_3 < \beta_4 < (\beta_5 = \beta_2) < \beta_1 < \beta_6$	{{3,4},{5,2},{1},{6}}	{{3},{4},{5,2},{1},{6}}	-0.269	0.5083	0.2554
		{{3},{4,5,2},{1},{6}}		-0.242	-1.3648	0.0248
		{{3},{4},{5,2,1},{6}}		**-0.221**	**0.7488**	**0.3673**
		{{3},{4},{5,2},{1,6}}		-0.187	-2.1250	0.0104
3	$\beta_3 < \beta_4 < (\beta_5 = \beta_2 = \beta_1) < \beta_6$	{{3,4},{5,2,1},{6}}	{{3},{4},{5,2,1},{6}}	**-0.269**	**0.1019**	**0.2554**
		{{3},{4,5,2,1},{6}}		-0.236	-3.9934	0.0021
		{{3},{4},{5,2,1,6}}		-0.205	-3.8349	0.0025
4	$(\beta_3 = \beta_4) < (\beta_5 = \beta_2 = \beta_1) < \beta_6$	{{3,4,5,2,1},{6}}	{{3,4},{5,2,1},{6}}	-0.243	-7.3652	0.0001
		{{3,4},{5,2,1,6}}		**-0.205**	**-4.4818**	**0.0025**

Table 4: The MLEs of coefficients and LL values from the fitted dose response functions for the Steps in the bottom-up procedure

Step	Order of slopes	Null model	Alternative model	$\hat{\beta}$	LL	p-value
1	$\beta_3 = \beta_4 = \beta_5 = \beta_2 = \beta_1 = \beta_6$	{{3,4,5,2,1,6}}	{{3,4,5,2,1},{6}}	(-0.243,-0.169)	-7.3652	0.00003
			{{3,4,5,2},{1,6}}	(-0.250,-0.187)	-6.5995	0.00001
			{{3,4,5},{2,1,6}}	(-0.259,-0.197)	-4.8660	0.00000
			{{3,4},{5,2,1,6}}	**(-0.269,-0.205)**	**-4.4818**	**0.00000**
			{{3},{4,5,2,1,6}}	(-0.289,-0.221)	-11.9331	0.00337
2	$(\beta_3 = \beta_4) < (\beta_5 = \beta_2 = \beta_1 = \beta_6)$	{{3,4},{5,2,1,6}}	{{3},{4},{5,2,1,6}}	(-0.289,-0.260,-0.205)	-.38349	0.25535
			{{3,4},{5,2,1},{6}}	**(-0.269,-0.221,-0.169)**	**-0.1019**	**0.00246**
			{{3,4},{5,2},{1,6}}	(-0.269,-0.227,-0.187)	-2.7719	0.06442
			{{3,4},{5},{2,1,6}}	(-0.269,-0.232,-0.197)	-3.8492	0.26064
3	$(\beta_3 = \beta_4) < (\beta_5 = \beta_2 = \beta_1) < \beta_6$	{{3,4},{5,2,1},{6}}	{{3},{4},{5,2,1},{6}}	**(-0.289,-0.260,-0.221,-0.169)**	**0.7488**	**0.25535**
			{{3,4},{5,2},{1},{6}}	(-0.269,-0.227,-0.212,-0.169)	0.5083	0.36726
			{{3,4},{5},{2,1},{6}}	(-0.269,-0.232,-0.217,-0.169)	0.4947	0.37538

Table 5: The MLEs (Standard Errors) of coefficients of the joint dose-response function from multiple exposures that Do Not Have Constant Relative Potency Factors

s	α	β	ρ_a	ρ_b	ρ_c	ρ_d	ρ_e	w_a	w_b	σ	D	Pred$(D)^*$
1	-0.760	-0.221	1.032	1.600	0.437	0.273	4.718	1.215	0.762	0.171	0.2560	-0.4585
	(0.058)	(0.010)	(0.372)	(0.576)	(0.165)	(0.092)	(1.701)	(0.070)	(0.072)	(0.026)		
2	-0.767	-0.221	0.969	1.550	0.423	0.265	4.570	1.215	0.762	0.200	0.2479	-0.4585
	(0.057)	(0.010)	(0.349)	(0.559)	(0.159)	(0.088)	(1.649)	(0.070)	(0.072)	(0.031)		
5	-0.864	-0.221	0.625	0.645	0.273	0.171	2.949	1.215	0.762	0.306	0.1600	-0.4585
	(0.057)	(0.010)	(0.225)	(0.233)	(0.104)	(0.058)	(1.063)	(0.070)	(0.072)	(0.046)		
3	-0.577	-0.269	0.680	1.978	2.030	2.912	7.091	0.823	0.627	0.320	0.8810	-0.5427
	(0.058)	(0.010)	(0.176)	(0.597)	(0.610)	(0.871)	(2.107)	(0.047)	(0.058)	(0.050)		
4	-0.473	-0.269	1.471	2.909	2.986	4.282	10.429	0.823	0.627	0.232	1.2958	-0.5427
	(0.041)	(0.010)	(0.380)	(0.759)	(0.775)	(1.112)	(2.725)	(0.047)	(0.058)	(0.025)		
6	-1.104	-0.169	0.131	0.136	0.242	0.044	0.024	1.312	1.594	0.247	0.0126	-0.3660
	(0.056)	(0.014)	(0.067)	(0.070)	(0.120)	(0.025)	(0.013)	(0.124)	(0.148)	(0.036)		

$*$ Natural logarithm of the predicted response.

Table 6: The estimated cumulative risks from the individual model and the two proposed dose addition approaches for the six chemicals

	Individual model	Dose-addition model	
Chemical	$P(d)$	$P(D)$	$P^a(D)$
1	0.0024	0.3748	0.7368
2	0.0021	0.2396	0.5353
5	0.0020	0.0666	0.1616
3	0.0031	0.0961	0.1397
4	0.0032	0.2544	0.3679
6	0.0027	0.0645	0.3052
sum	0.0155		

Table 7: The estimates of the POD, total exposure dose (d_{RPF}), predicted response (Pred(d_{RPF})), and cumulative risk (P(d_{RPF})) for the six chemicals from the RPF method

Chemical	POD	d_{RPF}	Pred(d_{RPF})	P(d_{RPF})
1	0.0327	0.1965	-0.4290	0.3169
2	0.0387	0.2324	-0.4455	0.2166
3	0.1782	1.0701	-0.5676	0.1079
4	0.1894	1.1370	-0.5164	0.2166
5	0.0315	0.1892	-0.4668	0.0695
6	0.0019	0.0114	-0.3480	0.0552