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Considerations for Developing a Dosimetry-Based Cumulative Risk Assessment Approach for Mixtures of Environmental Contaminants

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NOTICE

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ABSTRACT

For most of its history, the U.S. Environmental Protection Agency (U.S. EPA) has assessed risks based on individual contaminants and has often focused on one source, pathway or adverse effect. But in reality, the public is exposed to multiple contaminants from a variety of sources, and tools are needed to understand the resulting combined risks. In keeping with its continuing effort of developing science-based risk assessment, a major mission goal and challenge for the U.S. EPA has been underway for the development of cumulative risk assessment. The Office of Pesticide Programs (OPP), U.S. EPA, took the lead and conducted cumulative risk assessment on organophosphorus (OP) pesticides under the Congressional mandate of the Food Quality Protection Act (FQPA), addressing only chemicals with the same mode/mechanism of action. The present work is complementary to that of OPP. This report develops a framework to guide decisions whether to incorporate physiologically-based pharmacokinetic (PBPK) modeling into the cumulative risk assessment process. It is not restricted to FQPA applications and intends to guide the risk assessor through choosing to conduct and conducting a dosimetry based cumulative risk assessment, addressing the level of toxicant in the target tissue, rather than relying on exposure concentrations and the hazard index approach. This report includes data and values for several compounds to demonstrate the proposed approach by means of example. It is not intended to present an analysis of the internal exposures or risks from these mixtures. Some of the example chemicals (trichloroethylene, tetrachloroethylene) are the subject of ongoing U.S. EPA risk assessments, and risk values for other chemicals are those developed by ATSDR, rather than by the U.S. EPA. The main objectives are (1) to improve the science and risk assessment by moving the measure of dose from concentration in the environmental contact medium to inside the body (i.e., tissue dose rather than exposure dose); (2) to achieve this objective is via PBPK modeling; (3) to incorporate PBPK modeling into the cumulative risk assessment process and (4) to make the optimal use of *in vitro* techniques, including the use and application of human tissues. The proposed approach retains the 10-step process developed by the OPP; these are: (1) Identify Common Mechanism Group (CMG); (2) Identify Potential Exposures; (3) Characterize and Select Common Mechanism Endpoint(s); (4) Determine The Need For a Dosimetry-Based Cumulative Risk Assessment; (5) Determine Candidate Cumulative Assessment Group (CAG); (6) Conduct Dose-

Response Analyses and Determine Relative Potency and Points of Departure; (7) Develop Detailed Exposure Scenarios for All Routes and Durations; (8) Establish Exposure Input Parameters; (9) Conduct Final Cumulative Risk Assessment; and (10) Conduct Characterization of Cumulative Risk. In the present effort, however, the first five steps were grouped under Phase I (Initial Analysis) and the next five steps were grouped under Phase II (Dosimetry-Based Cumulative Risk Assessment). PBPK models are proposed to be developed for the components that are considered in Phase II, i.e., those that are in the CAG. This approach stresses the importance of initial analysis to eliminate those situations that do not warrant a PBPK-based approach to cumulative risk assessment thereby reducing the unnecessary expenditure of resources. The development of this framework/approach utilizes two model sets of chemical mixtures: Mixture 1 consists of 6 OP pesticides (methyl-parathion, parathion, chlorpyrifos, fenthion, diazinon, and fenitrothion) which share the same mode of action of inhibiting acetylcholinesterase (AChE); Mixture 2 consists of four chlorinated hydrocarbon solvents or volatile organic chemicals (trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, and chloroform) which have different modes of action on a variety of toxic endpoints. The wealth of information available for these chemicals was a prominent factor in their choice as two model chemical mixtures for this effort. The advantages of utilizing PBPK modeling in cumulative risk assessment and the incorporation of credible human tissue studies in PBPK modeling, as well as the methodologies involving the incorporation of interactive PBPK models are discussed. The intent of this document is to serve as a logical framework upon which to integrate information to decide whether to embark on technical and resource-intensive PBPK approaches to cumulative risk assessment.

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TABLE OF CONTENTS

Page

TABLE OF CONTENTS cont.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AL	Acceptable level
AUC	Area-under the concentration vs. time curve
BMD	Benchmark dose
BMDL	Lower bound benchmark dose
BMDS	Benchmark dose software
CAG	Cumulative Assessment Group
CE	Carboxylesterase
CHF	Chloroform
CMG	Common Mechanism Group
CNS	Central nervous system
CRA	Cumulative Risk Assessment
DBCRA	Dosimetry-Based Cumulative Risk Assessment
CYP	Cytochrome P450
E	Exposure
FQPA	Food Quality Protection Act
GST	Glutathione S-transferase
HI	Hazard Index
HQ	Hazard Quotient
IRIS	Integrated Risk Information System
LOAEL	Lowest-observed-adverse-effect level
MC	1,1,1-Trichloroethane
NCEA	National Center for Environmental Assessment
NOAEL	No-observed-adverse-effect level
OP	Organophosphorus
OPP	Office of Pesticide Programs
PBO	Piperonyl butoxide
PBPK	Physiologically-based pharmacokinetic
PD	Pharmacodynamic
PERC	Tetrachloroethylene
PK	Pharmacokinetic
POD	Point of departure
PNS	Peripheral nervous system
RfC	Reference concentration

LIST OF ABBREVIATIONS cont.

RfD	Reference dose
TCE	Trichloroethylene
TTD	Target Organ Toxicity Dose
U.S. EPA	U.S. Environmental Protection Agency
VOCs	Volatile organic chemicals
WOE	Weight of evidence

GLOSSARY

Acceptable Level: A measure of the acceptable level of human exposures. For cumulative risk assessment, reference values are typically used.

Aggregate Exposure: The combined exposure of an individual (or defined population) to a specific agent or stressor via relevant routes, pathways, and sources.

Aggregate Risk: The risk resulting from aggregate exposure to a single agent or stressor.

Chemical Mixture: Any set of multiple chemical substances that may or may not be identifiable, regardless of their sources, that may jointly contribute to toxicity in the target population. May also be referred to as a whole mixture" or as the "mixture of concern."

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

Common Mechanisms Group: The group of chemicals under evaluation that induce a common effect via the same mechanism of toxicity. The Initial Analysis begins with the identification of this group of chemicals.

Critical Organ/Effect: The effect or the organ that responds first as dose increases. The dose-response for the critical organ serves as the basis for establishing reference values (e.g., RfD Values).

Cumulative Assessment Group: The group of chemicals surviving the Initial Analysis, for which a dosimetry-based cumulative Risk Assessment will be conducted.

Cumulative Risk: The combined risks from aggregate exposures to multiple agents or stressors.

Cumulative Risk Assessment: An analysis, characterization and possible quantification of the combined risks to health or the environment from multiple agents or stressors.

Dosimetry-Based Cumulative Risk Assessment: Cumulative risk assessment undertaken using tissue concentrations, or internal measures of exposure, rather than external measures of exposure.

Hazard Quotient: The ratio of human exposure to reference values used to estimate the potential for non-cancer health effects. HQ = E/AL, where E is exposure and AL is an acceptable level of exposure (e.g., RfD value).

Hazard Index: The summed Hazard Quotient values for a given set of chemicals or responding tissues, depending on the analysis.

GLOSSARY cont.

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.

Initial Analysis: The first 5 steps of the process of developing a dosimetry-based cumulative risk assessment. This initiates with the identification of a common mechanism group and culminates with the analysis of pharmacokinetic data and a Hazard index analysis.

Interactions: Interactions among mixture components are demonstrated by responses that, when measured, differ from those predicted by additivity. Chemical interactions may occur due to commonalities in pharmacokinetic or pharmacodynamic processes.

Pharmacokinetics: The (study of the) distribution of chemical dosimetry in the body.

Pharmacodynamics: The process of developing a biological response to a chemical.

Point of Departure: The dose-response point that marks the beginning of a low-dose extrapolation. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (BMD), or a NOAEL or LOAEL for an observed incidence, or change in level of response.

Target Organ: Any organ adversely affected by chemical exposures.

Target Organ Toxicity Dose Approach: A cumulative risk approach quite similar to the Hazard Index Approach. The TTD approach differs in that includes dose-response data to estimate AL in the E/AL format for all responding organs, not just the critical organ.

Target Organ Toxicity Dose Values: Hazard quotient values developed for organs that are not the critical organ in the IRIS Assessment, or for which a reference value has not been formally established.

Weight of Evidence: A qualitatively useful adjunct to the Hazard Index approach that incorporates information available in binary combinations of chemicals. This approach is used to account for interactions among mixture components.

Definitions obtained from U.S. EPA (2000a, 2003c) and Mumtaz and Durkin (1992).

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1. INTRODUCTION

1.1. BACKGROUND

On July 3, 1997, the U.S. Environmental Protection Agency (U.S. EPA) Administrator, Carol M. Browner, and Deputy Administrator, Fred Hansen, jointly issued a memorandum entitled "Cumulative Risk Assessment Guidance - Phase I Planning and Scoping" to top U.S. EPA officials. The content of this memo, quoted below, provided the essence of the reasoning for cumulative risk assessment.

> As you are aware, the processes that EPA and others follow to assess environmental risk are of great interest to environmental professionals and to the public, and growing attention is being given to the combined effects of multiple environmental stressors. Consistent with this, EPA and others are asking more questions about the wider and more complex issues that define a cumulative approach to risk assessment. Today, we are providing guidance for all EPA offices on cumulative risk assessment. This guidance directs each office to take into account cumulative risk issues in scoping and planning major risk assessments and to consider a broader scope that integrates multiple sources, effects, pathways, stressors and populations for cumulative risk analyses in all cases for which relevant data are available. This assures a more consistent and scientifically complete Agency-wide approach to cumulative risk assessments in order to better protect public health and the environment.
>
> This approach provides a platform for significant advances in our scientific approach to assessing environmental risks. For most of our history, EPA has assessed risks and made environmental protection decisions based on individual contaminants—such as lead, chlordane, and DDT—with risk assessments for these chemicals often focused on one source, pathway or adverse effect. Today, better methods and data often allow us to describe and quantify the risks that Americans face from many sources of pollution, rather than by one pollutant at a time. We are increasingly able to assess not simply whether a population is at risk, but how that risk presents itself. In addition, we are better able in many cases to analyze risks by considering any unique impacts the risks may elicit due to the gender, ethnicity, geographic origin, or age of the affected populations. Where data are available, therefore, we may be able to determine more precisely whether environmental threats pose a greater risk to women, children, the elderly, and other specific populations, and whether a cumulative exposure to many contaminants, in combination, poses a greater risk to the public.
>
> Of particular importance are the right-to-know implications of this guidance, which requires that we build opportunities for citizens and other

stakeholders to understand our ongoing risk assessments, and to provide us with their comments. Our goal is to ensure that citizens and other stakeholders have an opportunity to help define the way in which an environmental or public health problem is assessed, to understand how the available data are used in the risk assessment, and to see how the data affect decisions about risk management.

Some Regions and Programs within the Agency are already making significant efforts to use integrated or cumulative risk assessment techniques, and this guidance both reflects those practices and makes them consistent across the Agency. The scope of integrated risk assessments often involves coordination across many program offices and statutory mandates for risk analysis; for example, those called for under the new safe drinking water and food safety laws. Therefore, this guidance calls for ongoing communication among risk assessors, risk managers, economists, engineers, and other technical experts within the Agency.

While we can more consistently take into account many new factors in this approach to risk assessment, many other potentially important factors are more difficult to include in our analyses, particularly the social, economic, behavioral or psychological factors that also may contribute to adverse health effects. These include, among others, such factors as existing health conditions, anxiety, nutritional status, crime and congestion. Assessment of these factors is often hampered by a lack of data to establish plausible cause-and-effect relationships; difficulties in measuring exposure, incidence and susceptibilities related to these risks; and few methods for assessing or managing these risks. This guidance does not address these factors. We expect, nonetheless, that this guidance will be updated as our understanding and experience develop; and, the Agency is focusing its research to improve our ability to incorporate these broader concerns into our cumulative risk assessments as new data and methods are brought forward.

Please take the steps needed to ensure that all major risks assessments undertaken in your area embrace this cumulative approach, so that we can better advise all citizens about the environmental and public health risks they face, and improve our ability to protect the environment and public health for the nation.

During his tenure as U.S. EPA Science Advisor and Chair, Science Policy Council, Dr Paul Gilman underscored the importance of cumulative risk assessment to the U.S. EPA in the following memo entitled “Framework for Cumulative Risk Assessment” to senior managers at U.S. EPA.

I am very pleased to present EPA's Framework for Cumulative Risk Assessment. For most of our history, EPA assessed risks based on individual contaminants and often focused on one source, pathway or adverse effect. But in reality, the public is exposed to multiple contaminants from a variety of sources, and tools are needed to understand the resulting combined risks. The Framework represents an important milestone for EPA in expanding our focus from an individual chemical-based approach to a community or population-based approach for multiple stressors.

Development of the Framework is one of a series of Agency activities to better address combined risks from multiple stressors. Several EPA Programs and Regions have growing experience in conducting cumulative risk assessments, such as in evaluating cumulative risk for pesticides with a similar mode of action. In addition, the EPA Science Policy Council (SPC) has sponsored several efforts designed to lead to Agency-wide guidance on planning and scoping for cumulative risk assessments. As a next step, a technical panel of the Risk Assessment Forum has now completed the Framework, which builds on prior efforts by identifying the basic elements and definitions for cumulative risk assessment. It also serves as the foundation for future efforts, such as evaluating past and emerging case studies in relation to the approach outlined in the document. I offer my thanks to this cross Agency panel of experts for their efforts to further advance the thinking in this area.

Cumulative risk assessment is a major challenge for the Agency. This Framework moves us closer to achieving our goal of producing the most scientifically rigorous and realistic evaluation of cumulative risk that the state-of-the-science can accommodate. The Administrator and I encourage Agency personnel to incorporate the thinking embodied in this document in the development of cumulative risk assessments that address risk management needs.

1.2. STRATEGY: INCORPORATION OF PBPK MODELING AND HUMAN TISSUE STUDIES IN CUMULATIVE RISK ASSESSMENT

The present report follows the vision outlined in the above memos by proposing an approach which integrates Physiologically-Based Pharmacokinetic (PBPK) modeling, particularly the inclusion of credible human tissue studies, into the cumulative risk assessment of chemical mixtures. There are several reasons for utilizing the PBPK modeling approach:

1. As indicated in the above memos, U.S. EPA continues to improve its risk assessment process by incorporating the state-of-the-art science and technology. PBPK modeling, though advancing continuously in the last 30 years

or so, is a new computational toxicology technology. From a different perspective, the state-of-the-science is such that PBPK modeling is ready to be incorporated into cumulative risk assessment. In this regard, it is gratifying to note that an inter-office endeavor on "Physiologically-Based Pharmacokinetic/ Pharmacodynamic Modeling: Preliminary Evaluation and Case Study for the N-Methyl Carbamate Pesticides: A Consultation" at the U.S. EPA was reviewed by the FIFRA Science Advisory Panel in December 2003. (http://www.epa.gov/oscpmont/sap/2003/index.htm). However, due to the lack of availability of a PBPK model for all but one chemical, a PBPK modeling approach was not taken in the final assessment (U.S. EPA, 2007a). Many advantages, which coincide with what's presented here, were noted in the "Consultation" above document (U.S. EPA, 2003a) and the subsequent FIFRA SAP meeting minutes (U.S. EPA, 2003b) for utilizing PBPK modeling in cumulative risk assessment.

2. Multiple chemical exposure and interaction in our body is an extremely complex phenomenon. PBPK modeling is a powerful tool to integrate the various routes and modes of exposure and potential biological interactions with multiple chemicals to help resolve the best target tissue dose metrics for risk assessment.
3. PBPK models support hypothesis testing through experimentation of evaluation using *in silico* methods and serves as a platform upon which to integrate existing data, and extend the efficient use of the available data. A well developed PBPK model is tested for accuracy in simulating existing study results, and if sufficiently able to do so, can be used to simulate other needed data to minimize animal usage that would otherwise be required for "unnecessary experiments". This is particularly relevant for the many animal toxicology experiments that would be required to evaluate multiple chemical interactions in a cumulative risk assessment.
4. PBPK models can extrapolate dosimetry across dose level, route, species, age, or gender, and can characterize the uncertainty in simulation results (which are derived from the accuracy of the model in simulating existing data). A PBPK model may even provide useful information for the dose-response relationship at low doses where experimental studies are impossible to conduct.
5. PBPK modeling offers a reliable tool for use in exploring the relationship between external dose and tissue response in the growing area of systems biology.

1.3. OBJECTIVES

The main objectives/emphases are: (1) to improve the science and risk assessment by moving the measure of dose from concentration in the environmental contact medium to inside the body (i.e., tissue dosimetry rather than exposure dose); (2) to achieve the objective of tissue dosimetry determination via PBPK modeling; (3) to incorporate the best and most efficient scientific approach, PBPK modeling, into cumulative risk assessment; and (4) to make the best use of *in vitro* techniques;

including the use and application of human tissues. The intent of this document is to serve as a logical framework upon which to integrate information to decide whether to embark on technical and resource-intensive PBPK approaches to cumulative risk assessment.

1.4. TWO MODEL MIXTURES OF DRINKING WATER CONTAMINANTS

It should be emphasized that this effort is for establishing a framework/approach for integrating PBPK modeling into the cumulative risk assessment process, not the actual conduct of cumulative risk assessment. Indeed, two example component chemicals, trichloroethylene and tetrachloroethylene, are the subject of ongoing U.S. EPA risk assessments. The actual conduct of a cumulative risk assessment is understandably much more resource-intensive. As shown below, this report provides a description of a recommended approach for integrating PBPK modeling into cumulative risk assessment for two groups of drinking water contaminants. The first group is a mixture of six organophosphorous (OP) insecticides (methyl-parathion, parathion, chlorpyrifos, fenthion, diazinon, and fenitrothion) which share the same mode of action of inhibiting acetylcholinesterase (AChE); the second group is a mixture of four chlorinated hydrocarbon solvents or volatile organic chemicals (trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, and chloroform) which have different modes of action for a variety of toxic endpoints. We understand that chlorpyrifos and diazinon household usage have been cancelled; however, all these OP pesticides can still be in drinking water and/or as food contaminants because of residues from earlier applications. Furthermore, we use these two model mixtures as a test set for demonstrating the establishment of a framework/approach. The risk assessment approach is basically similar to the 10-step approach recommended by the U.S. EPA for cumulative risks (U.S. EPA, 2002a,b).

1.5. PHARMACOKINETICS vs. PHARMACODYNAMICS

Pharmacokinetics (PK) and pharmacodynamics (PD) are inseparable in the sense that they formulate a continuum of a toxicological process from exposure, to tissue dose metrics, to molecular interaction(s) and finally to toxic effect(s). This document will address the grouping of chemicals according to similarities in pharmacokinetics, pharmacodynamics (mode/mechanism of action) and target organ. These are mutually separable groupings: chemicals may attack the same target organ through different modes of action and have markedly different pharmacokinetic profiles with different physiological and or biochemical functions determining the

pharmacokinetic profile. Alternately, chemicals may share the same target organ, and may have similar pharmacokinetic profiles, but act on different cellular or organ function via different modes or mechanisms of action. Presently, efforts are advancing to guide the assessment of chemicals grouped by mode or mechanism of action, and mature guidance exists for grouping chemicals according to common target organ (ATSDR, 2004; U.S. EPA, 1989, 2000a). Because of the rate at which pharmacokinetic analyses are becoming directly and quantitatively incorporated into risk assessments, it seemed valuable to develop a discourse on some considerations that should be undertaken prior to embarking on a complex and resource-intensive pharmacokinetic analysis to support a cumulative risk assessment. For those reasons and because of the state of development of the area of PBPK modeling, we place more emphasis on PBPK modeling in this effort. Thus, at the present time, we would like to focus on incorporating PBPK modeling into the cumulative risk assessment process first. As the science advances and more relevant information becomes available, we will consider PBPD modeling in cumulative risk assessment as well.

Because cumulative risk assessment will impact upon our society at large, when this process is carried out, it should be transparent and inclusive of all stakeholders much the same way as Office of Pesticide Programs (OPP) did it for OP pesticides (U.S. EPA, 2002b, 2003c).

1.6. INTERACTIONS: CUMULATIVE RISK AND PHARMACOKINETICS

The term “interaction” has different meanings regarding cumulative risk and pharmacokinetics. In cumulative risk parlance, interaction describes the risk outcome (toxicity, organism response) when exposure to a chemical mixture results in a degree of response not predicted by additivity (e.g., antagonism, synergism, potentiation). If the outcome is over or under-predicted by additivity, then the chemicals are said to have an “interaction.” With respect to applying an increased understanding of tissue dosimetry in risk assessment, assessing interactions becomes problematic when the observed response is related to the external dose (mg/kg) and the observation (i.e., enzyme levels in blood indicative of heart damage) is made at the level of the intact organism. The outcomes are influenced by absorption, distribution, metabolism and elimination processes (pharmacokinetics) on production/reduction of the toxic chemical species and its delivery to the target organ/tissue, as well as the inherent responsiveness of the tissue to the insult (toxicodynamics).

Later, this document will present a grouping of chemicals into a Common Mechanism Group, which is done so that a cumulative risk assessment can be

performed on that group. Recently, the organophosphate insecticides have been grouped according to their common inhibition of acetylcholinesterase (AChE). This is the key step in their toxic effect—for which the mode of action might be an accumulation of acetylcholine. The distinction between mechanism of action and mode of action can be important and has been treated elsewhere:

> The U.S. EPA Guidance for Conducting Health Risk Assessment of Chemical Mixtures (U.S. EPA, 2000) defines mode of action (MoA) as a series of 'key events' and processes starting with interaction of an agent with a cell, and proceeding through operational and anatomical changes causing disease formation. A 'key event', as defined in the 2005 U.S. EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), is an *empirically observable precursor step that is itself a necessary element of the mode of action* or is *a biologically based marker* for such an element. In contrast, mechanism of toxic action implies a more detailed understanding and description of events, often at the molecular and cellular level (U.S. EPA, 1999, 2003a). The U.S. EPA 2005 and U.S. EPA 2000 Guidelines both emphasize MoA over mechanism, indicating that MoA is a critical determinant for evaluation of risk posed by environmental hazards. For well defined modes of action, mechanistic data may merely provide more detail in support of identified key events. However, for less well defined toxic modes of action, mechanistic data may lead to identification of previously unknown obligatory steps in the causal pathway leading to toxicity (Lambert and Lipscomb, 2007).

What is most important is whether chemicals in a mixture can interact, altering the toxicity of the mixture. Later, this section presents several examples of chemical interactions that would not have been anticipated based only on data describing either the mode of action or the mechanism of action.

From a pharmacokinetic standpoint, chemicals have the potential to interact in either passive (i.e., distribution phenomena; tissue partitioning) or active (i.e., energy-requiring processes; active transport, metabolism) processes. Available evidence indicates that chemicals partition into tissues independent of one another, when encountered at concentrations that do not disrupt tissue integrity. However, it is widely accepted that chemicals (e.g., metabolic substrates) can, and do compete against one another for metabolism, and that the likelihood for this competition increases with increasing levels of exposure (dose). When substrates are metabolized by the same enzyme, or require the same cofactor, interaction is possible. This interaction may be based on a suicide inhibition of an enzyme (mechanism based inhibitors) or a simple competition for an enzyme (competitive inhibition). Mechanism based inhibitors (suicide

substrates; this type inhibition is demonstrated as non-competitive) have effects that cannot be overcome by changing the ratio of substrates. When these substrates are metabolized, an irreversible complex is formed between product (or intermediate) and enzyme, irreversibly binding the active site and inhibiting catalysis. However, competitive inhibition is more common. Even chemicals (competing substrates) that share the same enzyme may not demonstrate inhibition under some conditions (levels) of exposure. Studies have demonstrated that there are thresholds of exposure, below which chemicals that do share a common enzyme do not interfere with the metabolism of one another. In the case of chemicals thought to have a metabolic (competitive) interaction, the total exposure to the mixture, rather than the exposure to single components should be evaluated. Dobrev et al. (2001) constructed an analysis to estimate interaction thresholds in the rat for three commonly found environmental contaminants, trichloroethylene (TCE), tetrachloroethylene (PERC) and 1,1,1-trichloroethane (methyl chloroform; MC). PBPK models were constructed for each chemical using published values for parameters, including K_m values. PBPK modeling of gas uptake data was applied, and interactions were characterized as concentrations of PERC and MC required to increase blood TCE concentrations by 10%. These solvents were chosen because of their ubiquitous occurrence as environmental contaminants, as well as their dependence on cytochrome P450 (CYP)-mediated oxidation as a primary metabolic process. Interaction models were constructed for competitive, non-competitive and uncompetitive inhibition. Although all models fit the data to some extent, the similarity between model-predicted (optimized) values for the inhibition constant (K_i) and K_m values indicated that the competitive model best fit the data. This model was implemented to interpret gas uptake data for binary and ternary mixtures. In the model, TCE exposure was maintained at the TLV of 50 ppm and interactions from binary and ternary exposures were examined. Under binary exposures to TCE and either MC or PERC, the threshold for interaction was 175 ppm (MC) and there was no interaction when PERC exposure was at 25 ppm. However, when the model simulated a ternary exposure to TCE (50 ppm), PERC (25 ppm) and MC, interaction was noted at MC concentrations of 130 ppm (compared to 175 ppm when encountered in a binary mixture). These results demonstrate the value of PBPK modeling in refining the descriptions of toxicologically important (metabolic, toxicokinetic) interactions among components of environmentally-important mixtures.

The above interaction is metabolic, or pharmacokinetic in nature. When a mixture or cumulative exposure results in a response different from that predicted from additivity and there are no data on tissue dosimetry, it is not possible to determine

whether the interaction is based on toxicokinetic or toxicodynamic interactions, or both. From an “interactions” perspective, it is possible that pharmacokinetic interactions may explain chemical mixture (cumulative risk) interactions, that may not be “interactions” as defined by cumulative risk; such departures from additivity may only be departures when considered at the level of the external or applied dose, and may be strictly additive when “dose” is expressed as “dose metric”—the concentration of the toxicologically active chemical species in the target tissue.

For example, consider two chemicals, A and B, that share a common metabolic pathway. The response from chemical A is dependent on a metabolite, whereas the response from chemical B is mediated by the parent compound. When the animal is concomitantly exposed to these chemicals at a level where metabolic interactions do occur—chemical A out-competes chemical B for metabolism; chemical A is metabolized to a greater extent than chemical B, the response from a mixtures exposure is much higher than the response predictions made on the assumption of additivity applied to single chemical toxicity results. An explanation may include the metabolic interaction, and when the “dose” is expressed in terms of the biologically active dose (a metabolite of chemical A and the parent form of chemical B), the response may, indeed, be predicted by additivity. Appreciating, developing and communicating the distinction, as well as its basis, will decrease the level of uncertainty associated with assessments of cumulative risk.

Pharmacodynamics is concerned with the development of the response, ultimately at the level of the cell. Processes that determine or modify delivery of the toxicologically active chemical species to the cell are addressed as pharmacokinetics. The biological response is ultimately mediated at the cellular level and key events may be identified, and sorted into those defining the mechanism of toxicity and those defining the mode of action. However, when chemicals act on the same biological and/or biochemical cellular processes, a toxicodynamic interaction may occur, regardless whether the shared biological processes are involved in mode or mechanism of toxic action. These are not trivial, and interactions may occur in biochemical pathways not directly involved in the critical toxic event for a chemical. Piperonyl butoxide (PBO) is a synergist in some insecticides. PBO is not especially toxic, and it inhibits enzymes that are important in detoxicating insecticides. Under a single-chemical exposure to PBO, inhibition of these enzymes is not especially important, though it does represent a toxicodynamic effect—it represents an adverse interaction of the toxic agent with a biomolecule. However, when PBO is co-exposed with an insecticide, the result is rather marked potentiation of insecticidal activity. The

interaction is toxicokinetic in this regard, because the potency (when measured as effect per applied dose) of the insecticide is increased. However, if the measure of dose is expressed relative to the internal dose of the insecticide, the potency (expressed per mg/kg of internally circulating insecticide) is not altered. An example of a strictly toxicodynamic interaction may occur when two chemicals share a common biological or biochemical event. If both chemicals alter cellular communication resulting in an outgrowth of damaged cells, then this type of interaction is likely to demonstrate an effect characterized by additivity. This is the type of interaction that is anticipated for mixtures of organophosphate insecticides. However, if two chemicals act on different biochemical processes in the same response pathway, a response other than additive might occur. Such would be the case if one chemical caused a decrease in the efficacy of DNA repair and/or a release of apoptotic control of cellular growth, and a second chemical caused damage to DNA. Under the event of a co-exposure, then the net result might be the outgrowth of cells in which DNA damage was responsible for toxicity. The effect resulting from the DNA damage might be a further loss of control over cell growth leading to tumorigenesis, or may be manifest as the production of a protein whose function has been adversely affected, resulting in increased cellular toxicity.

Whereas some of the above interactions results in potentiation or synergism, such is not always the case. Mehendale and colleagues evaluated the interaction between thioacetamide and carbon tetrachloride (reviewed in Mehendale, 2005). Both compounds are known to induce liver injury. However, it was demonstrated that low doses of thioacetamide given in the hours before carbon tetrachloride administration actually protected against CCl_4-induced hepatotoxicity. Ultimately, the effect was demonstrated to reside in the rebound effect of cellular repair following thioacetamide exposure. Here, the pre-exposure resulted in a stimulation of cellular repair processes; these processes were already active when CCl_4 exposure and injury occurred. The interaction was significant to the extent that thioacetamide exposure could spare animals from doses of CCl_4 that would otherwise have been lethal (reviewed in Mehendale, 2005).

The application of pharmacokinetic analyses to chemical interactions can suggest the basis for interactions (determine whether and to what extent departures of response from additivity can be attributed to pharmacokinetic interactions). An example is provided by the joint exposure of kepone and carbon tetrachloride. Mehendale and colleagues have demonstrated a significant interaction between these chemicals, resulting in an approximate 67-fold induction of CCl_4-induced hepatotoxicity. A PBPK model was developed to study the interactions between CCl_4 and kepone in rats

(El-Masri et al., 1996a). Previous experimental findings demonstrated that kepone co-exposure amplified the CCl_4-induced lethality 67-fold. In an additional study, CCl_4 was administered i.p. to rats fed a control diet or a diet containing Kepone. Data describing the pathology and the exhalation of CCL4 (indicative of metabolism) were collected and a pharmacodynamic model was developed and incorporated that characterized the injury, repair and death sequence for cells and animals. The results of this PBPK-PD model demonstrated an approximate 61 to 142-fold increase in CCl_4 lethality with kepone treatment. However, when the results were adjusted for PK differences in CCl_4 metabolism, the increase was approximately 4-fold. These results suggest that the kepone-CCl_4 interaction is primarily based on a TK interaction, but may also rely to some extend on a TD interaction.

Another example of pharmacokinetic interactions altering response can be provided by the organophosphate insecticides. While these compounds have been compiled into the same common mechanism group based on inhibition of AChE (the toxicodynamic determinant of CNS toxicity), a pharmacokinetic interaction among OPs may also influence response. These compounds are degraded by carboxylesterase (CE) enzymes. When exposure to OPs includes some compounds with relatively low toxic potency (e.g., fenitrothion) as well as more potent compounds (like parathion), the lower potency compounds may compete against the other compounds for detoxication via CE enzymes. The net result can be that higher levels of internal dose per unit external dose are developed than would have been predicted on the basis of single chemical studies (see Chambers et al., 1991; Cohen, 1984). In this example, interaction would occur at a level that may not have been predicted based on the results of toxicity studies. In this regard, this case is similar to that demonstrated by piperonyl butoxide: metabolic interactions alter internal dosimetry and are responsible for toxic interactions.

This example highlights another important consideration—that of timing. Timing may relate to pharmacokinetics, where dose timing can be different when characterized at the level of the external (encountered) dose or the tissue dose. For some chemicals, a prolonged tissue exposure can result from a brief environmental contact. In those instances, toxicant concentrations in tissues can remain appreciable hours, days or even weeks after the cessation of external exposures. Here, a joint exposure might occur, even if the person is not concomitantly “exposed” to multiple chemicals. Another mechanism through which timing may be complicated is when the effects of an exposure persist following cessation of exposure and elimination of the compound from the body. An example of such an effect is provided by the enzyme inducing ability of

some compounds. Many compounds can and do induce hepatic cytochrome P450 enzymes, and the previous administration of these compounds can modify the toxic (or therapeutic) response to subsequently-encountered xenobiotics via a modification of their internal dosimetry. The metabolism of a compound may represent a bioactivation or a detoxication process, and so the effect of enzyme induction (or inhibition) on toxic outcome must be evaluated on a chemical by chemical basis.

2. THE PROPOSED APPROACH

2.1. OVERVIEW

The existing U.S. EPA guidance for addressing the cumulative risk for pesticide mixtures acting through a common mode of action (U.S. EPA, 2002a)[1] suggests a series of evaluations that first lead to the determination of the Cumulative Assessment Group. The U.S. EPA's Office of Pesticide Programs has undertaken cumulative risk assessments for four groups of compounds (organophosphates, N-methyl carbamates, triazines and chloroacetanilides), which are available at http://www.epa.gov/pesticides/cumulative/common_mech_groups.htm. The reader of this report should not confuse the Cumulative Assessment Group (CAG) with the Common Mechanism Group (CMG). A cumulative risk assessment can include chemicals for which the mechanism(s) of toxicity may be similar (common) or independent. For the purpose of this document, the CAG is the group of chemicals that will undergo full risk analysis (the Dosimetry-Based Cumulative Risk Assessment; DBCRA) and may not include all chemicals originally considered. For some chemicals, data on exposures and/or dose-response may be so limiting, or a comparison of anticipated exposures to risk levels may reveal such a gulf that these chemicals would be "de-selected" for inclusion in a DBCRA. In some instances, this situation may be relevant to the entire mixture. For those chemicals or mixtures, only the conduct of a screening level analysis may be warranted. Therefore, steps prior to the determination of the CAG may be thought of as an "Initial Analysis" to determine whether the exposures to a chemical warrant the DBCRA. DBCRA is an analysis that includes evaluation of and inclusion of tissue or organ-specific levels of active toxic agent. Tissue levels of toxicant are employed in the determination of toxicokinetic interactions, i.e., metabolic competition, and tissue levels of toxicant are employed in the prediction of toxic events (toxicodynamics). In some cases, the Initial Analysis may be sufficient. For instance, the likelihood of a positive response predicted by additivity my not be of concern, and there may appear to be no reason to suspect an interaction (for example, if the Target Organ Toxicity Dose-based Hazard Index is appreciably below unity). If

[1] In 2002, the U.S. EPA, Office of Pesticide Programs (OPP) finalized guidance that would be relied upon to conduct a Congressionally-mandated assessment of the cumulative risks of organophosphate and other pesticides, including the risks to children. The guidance specifically addressed the development of an FQPA Safety Factor for the mixture of pesticides. The guidance document itself indicates that the intent of the document was not to serve as a guide for the conduct of all cumulative risk assessment approaches, within or without OPP. Specific provisions were included so that risk assessors may choose to depart from specific guidance when data and circumstances warrant doing so. This guidance has been subjected to Agency and Public review and is freely available on the World Wide Web.

there is insufficient evidence to further support a DBCRA, then the results of the Initial Analysis may be sufficient, and embarking on a DBCRA may not be deemed necessary. Alternately, the screening hazard index approach—that of assessing cumulative risks on the basis of external (instead of internal) concentrations/doses, may apply (U.S. EPA, 2000a). Thus, the hazard analysis of chemicals in a mixture during the "Initial Analysis" requires not only a determination of the population risk presented by the chemical alone, but any additional risks that would occur due to interactions in the mixture. The 10-step process communicated in this document (Figure 1) is divided into the two broad activities, Initial Assessment and the conduct of the Dosimetry-Based Cumulative Risk Assessment. In this process, the CAG is determined in step 5; steps 6-10 describe the specific activities that comprise the DBCRA.

A biologically based analysis of risks including evaluations of tissue dosimetry can be very resource-intensive and this is the main reason why the "Initial Analysis" is carried out to avoid investing resources into analyses that are not worthwhile. Likewise, development of PBPK models, particularly interactive PBPK models (PBPK models capable of addressing mixtures of chemicals, where additive and non-additive interactions at the level of pharmacokinetics and/or pharmacodynamics can be addressed) for chemical mixtures, would require additional resources. Thus, it is important to apply a screening method (initial analysis) to eliminate from further consideration and development of a DBCRA (a CRA whose exposure and dose response evaluations are based on tissue concentrations of toxicant, rather than on externally encountered concentrations/doses) those chemical mixtures anticipated not to cause significant cumulative risk and/or those mixtures for which the available data would not support a credible DBCRA. Accordingly, we recommend that the cumulative risk assessment (here, for drinking water contaminants) be conducted in a two-phase framework (1) Phase I: Initial Analysis; and (2) Phase II: Dosimetry-Based Cumulative Risk Assessment. PBPK models would be developed for the components that are considered in Phase II, i.e., those that are in the CAG. This approach stresses the importance of the initial analysis to eliminate those situations that do not warrant a full-scaled cumulative risk assessment thereby reducing the burden of the Public in conducting dosimetry-based cumulative risk assessment.

The 10 steps described in the U.S. EPA guidance document on cumulative risk assessment (U.S. EPA, 2002a) from the Office of Pesticide Programs remained unchanged under our proposed two-phase approach because: (1) the steps have been established, reviewed, and appear useful as a starting point, (2) we would

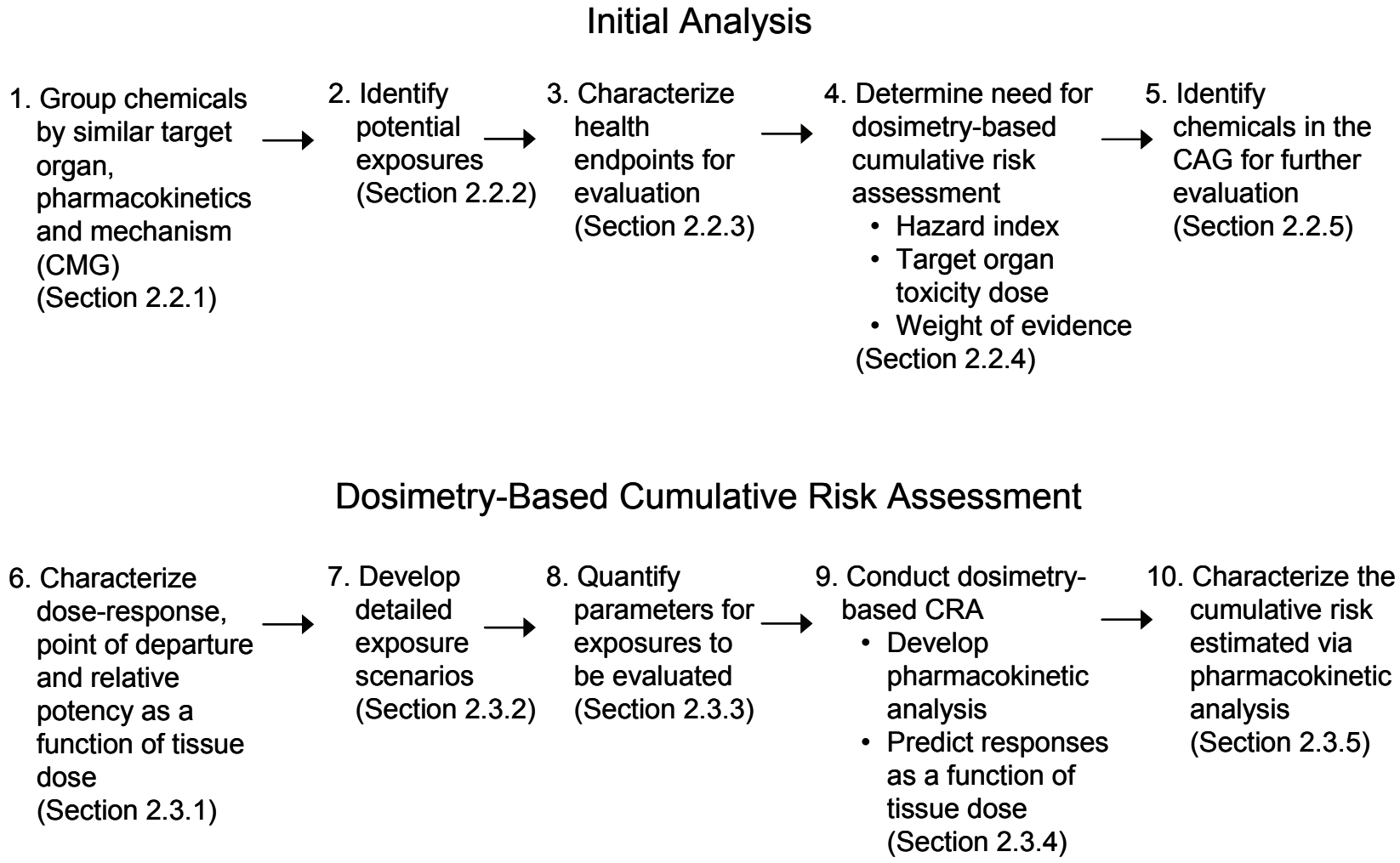


FIGURE 1

Data Evaluation for Dosimetry-Based Cumulative Risk Assessment

like to maintain consistency with the published U.S. EPA approach (U.S. EPA, 2002a); and (3) for any chemical mixture which is to undergo dosimetry-based cumulative risk assessment, it would have gone through the first five initial screening steps. Our framework/approach simply organizes the 10 steps into two phases to underscore the rationale for introducing PBPK modeling at the 6th step.

2.2. PHASE I: INITIAL ANALYSIS

In this phase, the principal purpose is to determine, from all available information on the chemical mixture and its exposure scenarios, whether a dosimetry-based cumulative risk assessment is warranted. Central to this determination or decision are the answers to the following questions:

- Is there the likelihood of toxicological interactions among the chemicals in the mixture? At what concentration levels?
- Are these toxicological interactions likely to cause higher toxicity (i.e., synergism or potentiation) or lower toxicity (i.e., antagonism) than the simple addition of the toxicity from all components of the mixture?
- Under the exposure conditions and levels, are these toxicological interactions possible?

2.2.1. Step 1. Identify Common Mechanism Group (CMG). A cumulative risk assessment begins with the identification of a group of chemicals, a Common Mechanism Group (CMG), that induce a common toxic effect by the same mechanism of toxicity. When chemicals are identified, data on their interactions should be sought. Two sources for such data are the general toxicological literature and the Interactions Profiles series developed by ATSDR (http://www.atsdr.cdc.gov/interactionprofiles/). An approach developed to guide OPP through conducting a mandated cumulative risk assessment of organophosphate pesticides (U.S. EPA, 2002a) refers to the definition of mechanism of toxicity from the Food Quality Protection Act (FQPA): “the major steps leading to an adverse health effect following interaction of a pesticide with biological targets.” However, this definition is somewhat ambiguous regarding whether the interactions between the chemical and the biological target are PK or PD in nature. Existing U.S. EPA guidance (U.S. EPA, 2002a) considers this issue in Step 3 of the CRA process. However, if a chemical is not included in the CMG group because it does not share relevant elements of PD processes with other chemicals in the group, but the chemical in fact can interact with other chemicals in the group through PK processes,

an underestimation (or overestimation) of the actual cumulative risk may occur. For example, suppose four chemicals (Chemicals A, B, C, Q) are being considered for inclusion in a CMG based on Effect X. Chemical Q does not cause Effect X, but Q is an inhibitor of metabolism of Chemical A, and this inhibition causes an increase in the Effect X for the same exposure to Chemical A (see also Section 1.6). If Chemical Q is omitted from the CMG due to lack of Effect X, the CRA will not properly characterize the cumulative risk for Chemicals A, B, C when Chemical Q is also present. For this reason, all chemicals that share common types of PK or PD interactions with biological targets should be considered as members of the CMG. This represents a specific extension of the process, as recommended by the available (OPP) guidance to address commonalities beyond those related to toxic manifestation: those pertaining to tissue doses of toxicologically active chemical species (metabolites). While OPP's guidance defines the CMG based only on toxicodynamic events in the mechanism of action itself, The CMG is here defined differently. Our definition is broadened to include commonalities or interactions in toxicokinetics that may alter tissue dosimetry and modify the toxic response. This has been done to optimize the application of PBPK modeling to assess cumulative risk.

For this project, the first mixture consists of 6 OP pesticides: methyl-parathion, parathion, chlorpyrifos, fenthion, diazinon and fenitrothion. For one effect of concern, neurotoxicity, the common mechanism of these pesticides is inhibition of AChE. Thus, the mechanism of toxicity includes a common pharmacodynamic process. However, many of these OP pesticides are also metabolized by common enzymes, primarily in the liver, including cytochrome P450s and esterases in various tissues. Therefore, this CMG includes chemicals that have similar pharmacokinetics, pharmacodynamics and target organs. During the CRA process for OP pesticides, the risk assessor will obviously also consider other toxicity endpoints, such as developmental neurotoxicity, before deciding which endpoint is most relevant. For at least some of the OP pesticides being considered here, there is some evidence that exposures during pregnancy or during childhood development may be important, because development is occurring and chemical may be transferred from the mother (e.g., placental transfer or via breast milk), because the child may have increased sensitivity, or because the child may exhibit pharmacokinetic differences from adults that lead to greater toxicity (Chanda and Pope, 1996; Qiao et al., 2001). Considerations of potentially susceptible subpopulations add to the complexity of PBPK modeling, when subpopulations exhibit anatomic, physiologic and biochemical differences compared to the general population.

The second mixture consists of four chlorinated hydrocarbons or volatile organic chemicals that are often present in drinking water as disinfection byproducts or groundwater contaminants: TCE, PERC, MC and chloroform (CHF). The mechanisms of toxicity for these chemicals are somewhat different depending on the type of toxic endpoints. For instance, at higher doses, particularly through inhalation exposure, all of these chemicals cause acute central nervous system (CNS) depression through the common mechanism of interference in ion channel function (Bruckner and Warren, 2001). In general, narcotic effects by solvents are caused by the following mechanisms: (1) membrane expansion and associated conformational changes of proteins (Shibata et al., 1991); (2) interaction with macromolecules (e.g., membrane lipids and proteins) and associated changes of membrane fluidity (Ueda and Kamaya, 1984) and (3) interaction with ligand gated ion channels (Franks and Lieb, 1994). Specific mechanisms for such effects by the TCE, PERC, MC and CHF have not been revealed yet. Since we are specifically interested in the exposure to contaminants found in drinking water, the likely exposure levels are low. Thus, acute neurotoxicity is highly unlikely and does not need to be considered as an endpoint. However, a hazard quotient for such an effect could be developed. For volatile compounds, exposure should also consider volatilization and inhalation of compounds during water use.

Long-term, low-level exposure to this mixture in drinking water is a highly likely scenario. Therefore, in addition to other chronic effects, the possible carcinogenic potential of this mixture as a critical effect should also be considered. In that sense, damage to cellular targets from reactive metabolites from this group of chemicals may be considered as a common mode of action. However, the specific targets of PD mechanisms that are the critical determinants of toxicity at low dose may vary from one chemical to another in this group. All of the four chemicals to be considered here share common elements of PK processes, namely shared metabolic pathways by CYP2E1, glutathione S-transferase (GST), and possibly other enzymes. These enzymes detoxify some of the volatile organic chemicals and their metabolites, while bioactivating others. Based on the discussion above, all four chemicals should be considered to be part of the CMG based on the potential for PK and/or PD interactions.

2.2.2. Step 2. Identify Potential Exposures. For each CMG member, evaluate proposed and registered uses and use patterns to identify potential exposure pathways (i.e., food, drinking water, residential) and routes (oral, inhalation, dermal). For this project, the primary exposure is through consumption of drinking water although the potential exists for inhalation and dermal exposure (i.e., during showers). Additionally,

pesticide (and other chemical) exposures from food intake and dust or residue through home application remains a potential. In this latter case, for instance, Whyatt et al. (2002) reported that, of the 72 pregnant women residing in northern Manhattan and South Bronx who underwent personal air monitoring for 48 hrs during their third trimester, all (100%) had detectable levels of three insecticides: chlorpyrifos, diazinon, and propoxur. Fenske et al. (2002) reported that chlorpyrifos was measurable in the house dust of all homes they monitored in a central Washington State agricultural community; children in these homes had detectable urinary metabolite of chlorpyrifos. Here, if the DBCRA is to be conducted, the PBPK modeling has the advantage of being able to carry out computer simulations with a variety of exposure scenarios and assumptions with relative ease. This translates external concentrations to internal (target tissue) doses, representing a valuable advance in dose-response evaluation for animal studies, for species extrapolation of dose-response, and for the purpose of providing a more useful characterization of human exposure.

In Step 2, members of the CMG should be assessed for the potential for significant and/or overlapping exposures. Various sources of information on the range of levels of each chemical in public and private drinking water supplies should be consulted. When analyzing these data, the risk assessor will confront one of the conundrums to be encountered in this CRA: data on contaminants are often expressed only as ranges observed, with no information indicating which co-occur and which do not. A CRA can be based on these data, assuming that exposure to all of the chemicals coincide. However, it is likely that there is some correlation between the exposure data, such that some exposures do not coincide. Suppose that the concentration of Chemical A ranges from 0.01 to 1.2 mg/L in various drinking water supplies and that the concentration of Chemical B ranges from 0.005 to 1.3 mg/L, with no data available to demonstrate a correlation between the two. Should a worst-case CRA be performed at the upper limit of exposure data, or at some confidence interval based on those data? Such an approach would likely overstate the cumulative risk if, as is likely the case, the geographical coincidence of maximal exposures is not complete. An alternate approach would consider using a geographical scenario-based approach. Such an approach could perform a CRA based on regional exposure data, such as was previously performed for OP pesticides (U.S. EPA, 2002b). A further alternative could take a point sampling approach. In this approach, specific risk assessment calculations could be performed based on exposure data at discrete locations or at discrete points in the concentration distributions (see US EPA, 2006). Statistical analysis of the results could then be performed on the CRA output to determine the percentages (and locations) of

the population that are above various hazard indices. This approach would involve an iterative approach; the information addressed in step 10 would communicate which subpopulations and to what extent their risk may be increased above a given hazard index. We note that these latter approaches are more calculation-intensive, and the ability of the risk assessor to perform this more detailed analysis would depend on resources. We also note that, to the extent that interactions between chemicals are found to occur, a non-PBPK modeling approach would have difficulty in accurately determining the cumulative risks. This would be due, primarily, to a lack of mechanism to address the interactions of chemicals within the body, which may occur between the portal of entry and the target tissue. However, once validated interaction-based PBPK models have been developed, it is a relatively simple matter to implement them for any number of exposure-concentration scenarios (including the point sampling approach); this could even be automated in such a way that a large exposure dataset could be evaluated.

The potential exposures to each component in the mixture under as many exposure scenarios as possible (primarily use of drinking water and showering) should be evaluated. This analysis should include exposure information that may vary by season and geographic location. A formalized approach, similar to the five-step process developed by ATSDR (2005) may be undertaken. This systematic approach includes: (1) identification of contaminant source(s), (2) evaluation of environmental fate and transport, (3) identification of point or area of exposure, (4) identification of exposure route(s) and (5) characterization of the exposed population.

2.2.3. Step 3. Characterize and Select Health Endpoint(s) for Evaluation. For each CMG member, evaluate common health effects and affected tissues and affected organs attributable to the common mechanism of toxicity across all exposure routes and durations of interest, determine the time-frames of expression for the common toxicity, and evaluate the quality of the dose-response data for each CMG member. Recommend endpoints/species/sex that can serve as a uniform basis for determining relative potency. In the case of the OP pesticide mixture, the most reasonable endpoint to use should be the inhibition of brain AChE (because they are neurotoxic through this mechanism). This endpoint should be correlated with clinical signs which are usually available in the toxicology literature. During this step, attention must be given to subpopulations or life stages that may be more susceptible to the common toxic effect and mechanism (here, inhibition of erythrocyte AChE activity). For example, infants and children may not have fully developed metabolic pathways for detoxifying or

bioactivating chemicals in a common mechanism grouping (U.S. EPA, 2002a). The importance of describing the potential increased sensitivity of infants and children is described in Executive Order 13045, and U.S. EPA's guidance is provided in *EPA's Rule Writer's Guide to Executive Order 13045: Guidance for Considering Risks to Children During the Establishment of Public Health-Based and Risk-Based Standards* (U.S. EPA, 1998).

The selection of endpoints requires a consideration, at least semi-quantitatively, of the time-course of biological effects so that overlapping pharmacokinetics can be considered. Thus, it is necessary to consider "biologically effective dose." This would represent the concentration of toxicant in the target tissue; and in the case of the drinking water disinfection byproducts and contaminant mixture, could be taken as the area-under the concentration vs. time curve (AUC) of the reactive species or related dose metrics.

Another issue to be determined is the choice of sex and age. Traditionally, risk assessments and PBPK models are most commonly developed for adult male humans. This is probably because of the preference for performing initial studies in male rodents (because the male may be a simpler model from an endocrine standpoint). However, U.S. EPA has been clear in its position that work should not stop with the male model and should be extended to females as well. Thus, the logical process may be as follows: determine the sex for which the most data are available to support the DBCRA (PBPK models). Initially develop PBPK models (and DBCRAs) for this sex. This would likely be the male model. Subsequently, extend this to female using as much female-specific data as possible and extrapolating from pooled or male data as required. Discuss uncertainty in this context, i.e., there may be more certainty with the initial sex selected and more uncertainty with the latter sex. Specific anatomic, physiologic and biochemical factors thought to differ among sexes should be identified. These issues apply to both the PBPK modeling and the DBCRA itself.

The same thoughts apply to the issue of age. At this stage, the PBPK models and the DBCRA would probably be applied to adults, and possibly to children, although it would certainly be preferable if other age groups could be considered as well. Again, both PBPK models and DBCRA analysis will probably be supported by more data for adults than for children. Therefore, the models and DBCRAs should be initially conducted for adults and subsequently extended to children. These issues (issues relating to potential differences in sensitivity among subpopulations) should be kept in consideration while determining the common mechanism endpoint.

2.2.4. Step 4. Determine the Need for a Dosimetry-Based Cumulative Risk Assessment. The relationship between exposure and acceptable exposure limits should serve as the trigger in the decision whether to proceed with conducting a more technically based dosimetry-based cumulative risk assessment (DBCRA). The guidelines established by the U.S. EPA (1986) describe a Hazard Index (HI) screening approach that represents the first step in this evaluation (U.S. EPA, 1989). In that approach, the exposure (E) and the acceptable level (AL) are determined for routes of exposure and expressed in the same units. That approach favors AL measurements (reference dose [RfD] and reference concentration [RfC] values) available on the U.S. EPA's Integrated Risk Information System (IRIS). However, in the absence of such values, other reliable values (e.g., ATSDR Minimal Risk Level values) may be relied upon, or values may be developed from original toxicity findings in the absence of more reliable information. A Hazard Quotient (HQ; E/AL) is determined for each component of the mixture and a Hazard Index for the mixture is determined as the sum of the HQ values. When a group of chemicals is targeted for a CRA, a HI should be developed for the entire group, and HI values should also be developed for each CMG, independently. A hypothetical example of a four chemical mixture (chloroform, trichloroethylene, 1,1,1-trichloroethane and tetrachloroethylene) is presented below in Table 1. The exposures are hypothetical, for demonstration purposes only.

This is a conservative approach, based on the assumption of dose additivity for the mixtures components, and unconstrained in that the AL values are not segregated by target organ or tissue. Although there is no "bright line," as HI values increase above 1.0, risk from the mixture is considered to increase. Given the conservative nature of the screening approach, if the HI is less than 1.0, additional methods to calculate HI should be considered. Subsequent to the development of this approach (U.S. EPA, 1986), additional works have suggested the employment of a weight of evidence (WOE) determination to evaluate the potential for toxicological interactions[2] (ATSDR, 2004; Mumtaz and Durkin, 1992; Mumtaz et al., 1998; U.S. EPA, 2000a); a WOE exercise is recommended by the U.S. EPA in its *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000a). The WOE serves as the basis for the decision whether component chemicals interact to alter (increase or decrease) the toxicity of other components of the mixture. When HI values are below 1.0, the WOE will serve as the basis for further consideration of the mixture, when the

[2] A Weight of Evidence Determination is a judgment reflecting the quality of the available information that categorizes the most plausible nature of any potential influence of one compound on the toxicity of another compound for a given exposure scenario (U.S. EPA, 2000a).

TABLE 1

Screening Hazard Index Approach

Chemical	Exposure (mg/kg-d)	RfD (mg/kg-d)	Hazard Quotient
Chloroform	0.0025	0.01	0.25
Tetrachloroethylene	0.005	0.01	0.5
Trichloroethylene[a,b]	0.0025	0.04	0.06
1,1,1-Trichloroethane[b,c]	0.05	0.5	0.1
Hazard Index:			0.91

[a] IRIS RfD values were not available.

[b] RfD value was not available on IRIS; it was developed from a chronic no-observed-adverse-effect level (NOAEL) value for renal effects (ATSDR, 1997a) and an uncertainty factor of 1000.

[c] RfD value was not available on IRIS at the time of this report's completion; it was developed from a chronic lowest-observed-adverse-effect level (LOAEL) value for body weight reduction (ATSDR, 1995) and an uncertainty factor of 1000.

HI value alone would suggest that further consideration is not warranted. Thus, developing a weight of evidence increases the basis for decision making. Some points that should be considered in developing the WOE include:

- Information on the chemical mixture or combinations of components that can inform the magnitude and direction of toxicologic interactions,
- Information on mode or mechanism of action of component chemicals, and
- Information on mode or mechanism of action of related chemicals.

While the U.S. EPA (2000a) has codified a technical approach to refining the HI by incorporating mathematical values for WOE and additional factors, incorporating such technical guidance here is beyond the scope of the intended exercise.

When the HI for the mixture exceeds 1.0 or when the HI is considered in light of the WOE and cannot without uncertainty be reduced to a value below 1.0, then the mixture should be subjected to a more technical estimation of HI, based on the Target Organ Toxicity Dose (TTD) approach (ATSDR, 2004; Mumtaz et al., 1997; U.S. EPA, 2000a). In this approach, effect levels for multiple organs are compiled and combined with uncertainty factors to develop TTD values for each organ system affected whether the effect is the critical effect or a secondary effect. These TTDs are analogous to RfD values. HQ values are derived, but the AL values can be either TTD values or RfD values for the most sensitive tissue or organ. HQ is determined as, E/TTD; HI values are developed for each organ, by summing the HQ values for each chemical, within the organ. This approach is more technical in that it incorporates organ-specific measures of toxicity, expanding considerations of toxicity from only the critical target to additional target systems, and it ensures that major systems affected are taken into account. In the tables that follow, chronic oral NOAEL values for effects were taken from ATSDR Toxicological Profiles for the four respective chemicals (notable exceptions are that the IRIS RfD values for chloroform and tetrachloroethylene, where liver was the critical target, were incorporated). Information on chronic NOAEL values were duration-adjusted (days/week) when necessary, and uncertainty factors (10, 100 or 1000) were employed to adjust for animal to human extrapolation, human interindividual variability and, in some cases, subchronic to chronic duration). Tables 2, 3, 4 and 5 present the derivation of TTD values for chloroform, tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane, respectively.

TABLE 2

Derivation of TTD Values for Chloroform (ATSDR, 1997b)

Target	Resp	CV	GI	Hemat	Musc	Liver	Kidney	Repro	b.w.
Species	mouse	dog	rat	human	rat	human	human	dog	rat, dog
NOAEL (mg/kg-d)	60	30	200	21	200	0.96	0.96	30	30
NOAEL Adjusted	51.4	25.7	142	21	143	0.96	0.96	25.7	30
Uncertainty Factors	100	100	100	10	100	10	10	100	100
TTD (mg/kg-d)	0.51	0.26	1.4	2.1	1.4	0.10	0.10	0.26	0.30

Resp = respiratory system
CV = cardiovascular
GI = gastrointestinal
Hemat = hematological
Musc = musculoskeletal
Repro = reproductive system
b.w. = body weight reduction

TABLE 3

Derivation of TTD Values for Tetrachloroethylene (ATSDR, 1997c)

Target	Resp	CV	GI	Hemat	Musc	Liver	Kidney	Repro	b.w.
Species	rat	rat	rat	human		rat	mouse*		rat
NOAEL (mg/kg-d)	941	941	941	NR	NR	20	386	NR	941
NOAEL Adjusted	672	672	672			14.3	275		672
Uncertainty Factors	100	100	100			1000**	1000		100
TTD (mg/kg-d)	6.7	6.7	6.7			0.01	0.28		6.7

* LOAEL

** IRIS UF = 1,000. 10 each for UF_S , UF_A , UF_H

NR = not reported

Additional abbreviations are listed with Table 2.

TABLE 4

Derivation of TTD Values for Trichloroethylene (ATSDR, 1997a)

Target	Resp	CV	GI	Hemat	Musc	Liver	Kidney	Repro	b.w.
Species	rat	rat	rat		rat	rat	rat	rat	rat
NOAEL (mg/kg-d)	250	250	250	NR	250	250	50	NR	250
NOAEL Adjusted	179	179	179		179	179	35.7		179
Uncertainty Factors	1000	1000	1000		1000	1000	1000	1000	1000
TTD (mg/kg-d)	0.18	0.18	0.18		0.18	0.18	0.036	0	0.18

NR = not reported
Additional abbreviations are listed with Table 2.

TABLE 5

Derivation of TTD Values for 1,1,1-Trichloroethane (ATSDR, 1995)

Target	Resp	CV	GI	Hemat	Musc	Liver	Kidney	Repro	b.w.	Immuno/ Lymphat
Species	rat	rat	rat	rat	rat	rat	rat	rat	rat	rat
NOAEL	1500	1500	1500	1500	1500	1500	1500	1500	750*	1500
NOAEL Adjusted	1071	1071	1071	1071	1071	1071	1071	1071	536	1071
Uncertainty Factors	100	100	100	100	100	100	100	100	1000	100
TTD	10.7	10.7	10.7	10.7	10.7	10.7	10.7	10.7	0.54	10.7

* LOAEL
Additional abbreviations are listed with Table 2.

Employing TTD values, rather than simply relying on RfD values gives a more complete treatment. For example, liver was the critical target for chloroform and trichloroethylene, but kidney and body weight gain were the critical endpoints for trichloroethylene and 1,1,1-trichloroethane, respectively. When the HI is calculated based on segregated values (i.e., the TTD approach), tissue dose-response information is more optimally employed. While liver served as the RfD values for two of the four compounds, the HI value changed from 0.91 under the screening approach (Table 1) to 0.77 for liver under the TTD approach (Table 6), reducing concern for toxicological interaction. This change was due to liver not being the most sensitive target for trichloroethylene or 1,1,1-trichloroethane. Further, the TTD based HI appears quite low for the other potentially affected organs/tissues, serving to focus attention on the liver as the site most likely affected. Again, a weight of evidence should be developed, but specifically for liver effects, to further refine the decision whether to proceed to a full DBCRA for this mixture. This should include the number and types of possible exposure scenarios in conjunction with the associated information on the concentration of contaminant in food and environmental media available. The NOAEL and LOAEL values for the common health endpoints and target tissues should be compiled. This evaluation may suggest that an initial analysis for the CMG will indicate that there is no risk concern for this group of chemicals and no further detailed assessment will be necessary. The available information may also suggest that expending resources on a dosimetry-based cumulative risk assessment may not be warranted.

One possible scenario is that, for some or all of the component chemicals in the mixture of interest, there is insufficient data or a lack of data. In this case, depending on the importance of the mixture of interest to public health, the U.S. EPA may decide: (1) there are insufficient data to conduct a cumulative risk assessment, and the component chemicals in the mixture would be assessed by single chemical risk assessment methods; or (2) new research initiatives are to be launched to fill the data gaps for the specific purpose of conducting dosimetry-based cumulative risk assessment for the mixture of interest.

A thorough evaluation of the toxicological literature for each chemical in the CMG must be performed. This may be performed in general accordance with risk assessment guidance for single chemicals because interactions between chemicals are considered separately. The type and quality of toxicological data should be assessed. Moreover, the data must be evaluated in relation to the species, gender and age of the animal as surrogates for humans. Ultimately, studies must be identified in which data describing doses and quantified responses are presented in sufficient detail to serve as

TABLE 6

Target Organ Toxicity Doses, Hazard Quotients and Hazard Indices

		Exposure	Effect								
			Resp	CV	GI	Hemat	Musc	Liver	Kidney	Repro	b.w.
Chloroform		0.0025									
	TTD		0.51	0.26	1.4	2.1	1.4	0.01*	0.096	0.26	0.3
	Hazard Quotient		0.0049	0.0097	0.0018	0.0012	0.0018	0.25	0.026	0.0097	0.0083
Tetrachloroethylene		0.005									
	TTD		6.7	6.7	6.7		0	0.01*	0.28		6.7
	Hazard Quotient		0.00074	0.00074	0.00074			0.5	0.018		0.00074
Trichloroethylene		0.0025									
	TTD		0.18	0.18	0.18		0.18	0.18	0.04		0.18
	Hazard Quotient		0.014	0.014	0.014		0.014	0.014	0.063		0.014
1,1,1-Trichloroethane		0.050									
	TTD		11	11	11	11	11	11	11	11	0.54
	Hazard Quotient		0.0047	0.0047	0.0047	0.0047	0.0047	0.0047	0.0047	0.0047	0.093
Hazard Index			0.024	0.029	0.021	0.0059	0.020	0.77	0.11	0.014	0.12

* Liver is the critical organ for chloroform and for tetrachloroethylene. RfD values were taken from IRIS (U.S. EPA, 2007b). All other chronic TTD values (NOAEL values) were taken from ATSDR toxicological profiles for the respective chemicals.

the basis for the dose-response relationship. If the PBPK modeling approach will ultimately be used, PK or PD studies will have to be identified that provide information on the dose-response of the animal where the response is expressed relative to appropriate markers of exposure. These types of data can be then transformed to determine the appropriate NOAEL or benchmark dose (BMD) for each chemical.

For the two mixtures considered in this project, as an illustration below, the three questions are answered and related discussions are provided.

- Is there the likelihood of toxicological interactions among the chemicals in the mixture? At what concentration levels?

As shown in Figures 2 and 3, respectively for the metabolic pathways of OP compounds (Mixture 1) and the drinking water disinfection byproducts and contaminants (Mixture 2), there are several possible pharmacokinetic interactions. All OP compounds share virtually the same metabolic pathways involving: (1) cytochrome P450 mediated oxidative desulfuration, dearylation, or dealkylation; (2) calcium-dependent hydrolysis of phosphoester bond; and (3) GST conjugation reactions. Similarly, there are interwoven metabolic pathways in the biotransformation of TCE, PERC, MC and CHF (see Figure 3). At the pharmacodynamic level, various oxon derivatives would certainly compete for the inhibition of brain AChE; thus, toxicological interactions are highly likely. Cancer and noncancer effects have been observed following exposure to the component chemicals of Mixture 2. During the CRA screening process, both cancer and noncancer effects, including reproductive effects, should be reviewed for potential (non-additive) interaction and significance. Three (TCE, PERC, CHF) of the four chemicals are thought to be carcinogenic at some doses; there is limited evidence that MC could be carcinogenic at this time. Thus, if cancer is the endpoint and MC is regarded as non-carcinogenic, the other three chemicals will be contributors to the overall risk, but the potential for MC to affect the carcinogenic potency of the other chemicals through PK interaction needs to be evaluated. The appropriate dose metrics for cancer risk assessment for TCE, PERC and CHF are not clearly established, but generally involve metabolites such as trichloroacetic acid or phosgene. There is limited information regarding potential pharmacodynamic interactions between these chemicals that can be addressed within a CRA. On the other hand, there have been several studies of pharmacokinetic interactions that will be useful (Dobrev et al., 2001, 2002; Haddad et al., 2000; Thrall and Poet, 2000). Indeed, for some of the mixtures of these four chemicals, PBPK interaction models exist (see below). A more likely scenario

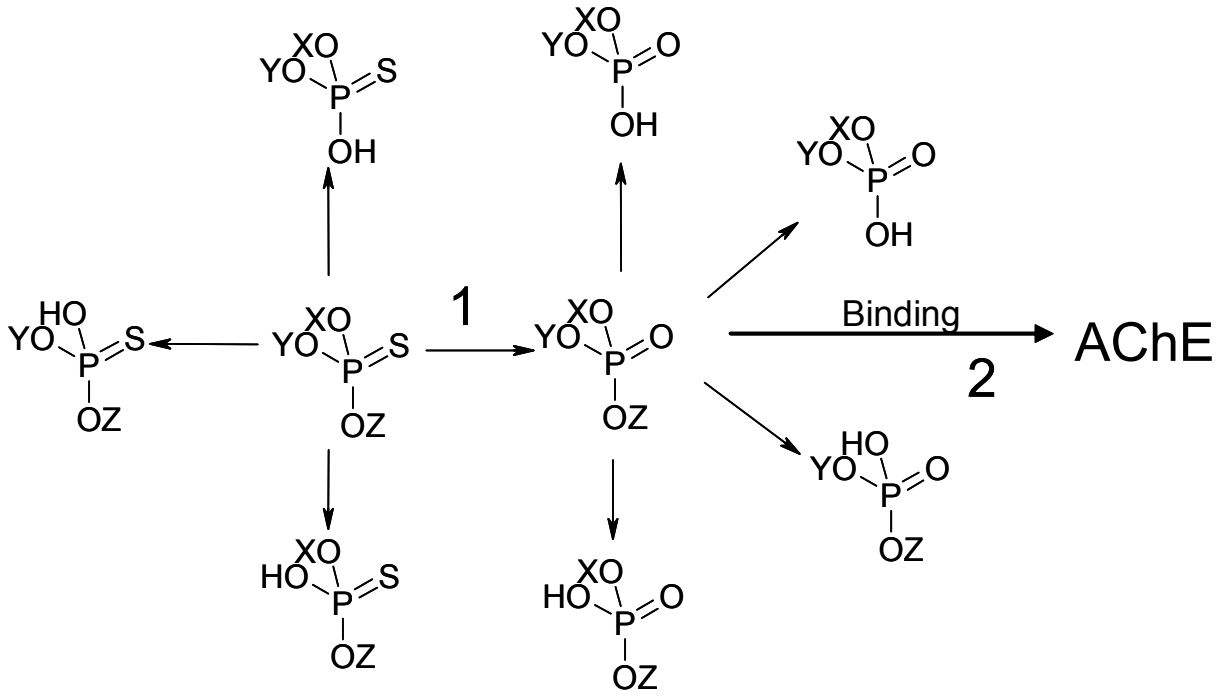
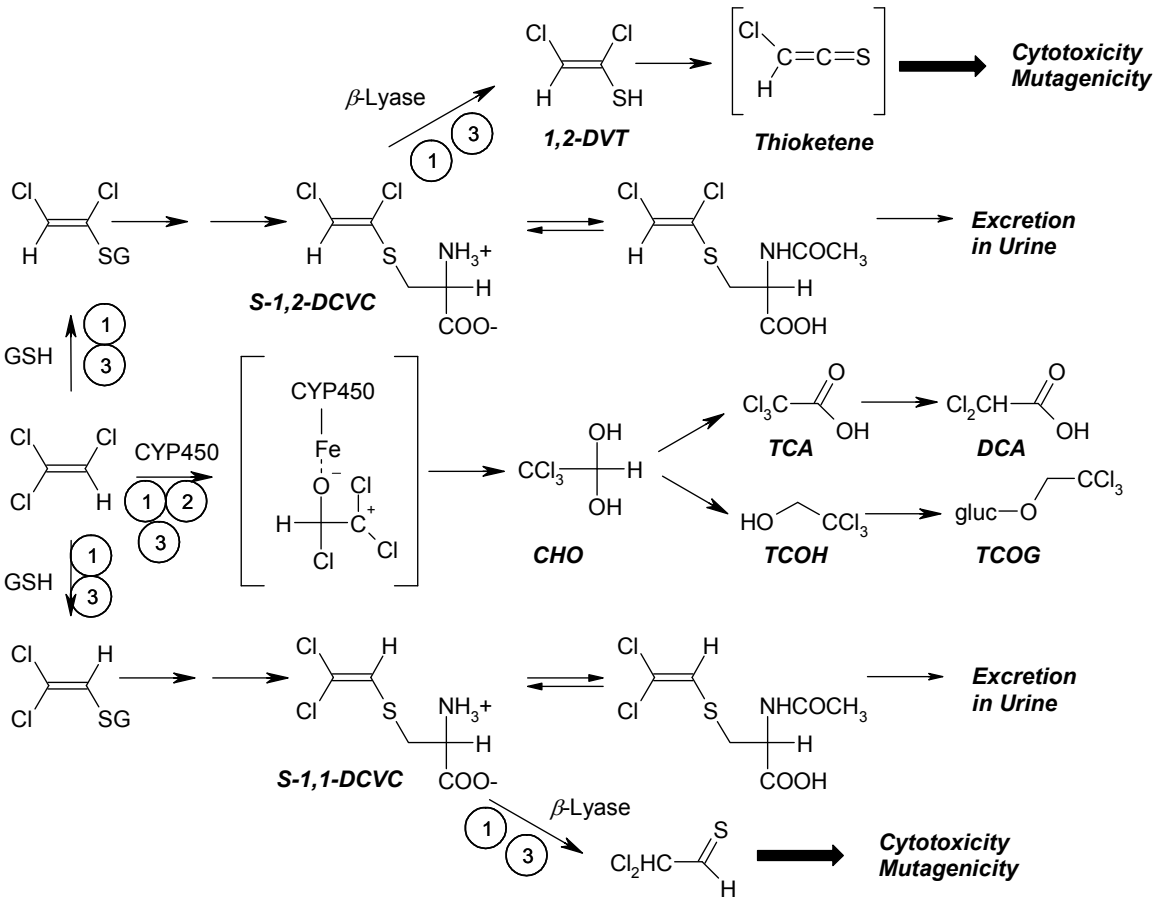


FIGURE 2

A Generalized Metabolic Schematic for OP Pesticides. Step 1 is activation and all other steps are detoxication. Pharmacokinetic interactions are most likely during Step 1. Interactions in other steps can be evaluated using PBPK models. Pharmacodynamic interactions may also occur during binding of the active moiety to AChE (Step 2).



(1) Potential interaction between PERC and TCE

(2) Potential interaction between MC and TCE

(3) Potential interaction between CHF and TCE

FIGURE 3

Metabolic Pathway for Solvents Indicating Potential Sites for Interactions. This is superimposed on a metabolic schematic for TCE (Dobrev et al., 2001), metabolic steps where pharmacokinetic interactions could occur are denoted by numbers as shown. Identification of additional potential interactions should occur during CRA Steps 3 and 6.

would be that some of the components could be excluded from the DBCRA (not from the cumulative risk assessment; for these chemicals, only a screening level assessment may be justified). It is important to avoid the expenditure of extensive resources to develop PBPK models for all components, only to find that the models indicate that a given component could have been omitted from the analysis based on a careful evaluation of data presented in the Initial Analysis.

As suggested by Figure 3, there are several common metabolic processes that overlap for TCE, PERC, MC and CHF. All are initially oxidized by Cytochrome P450, mostly by CYP2E1. Several studies have shown competitive inhibition at this step (Koizumi et al., 1982; Dobrev et al., 2001). Other steps are common to two or more of the chemicals, including metabolism by GST and β-lyase family enzymes. Potential interactions at these stages should be considered.

These interactions are likely to happen *in vivo* at the tissue levels of approximately nmolar to μmolar range which generally correspond to a mmole/kg administered dosing range (i.e., see Dobrev et al., 2002).

At this point, we believe that it is appropriate to address the toxicological interaction issue using a specific example reported in the earlier attempt of cumulative risk assessment of OP pesticides. In the G*uidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity* (U.S. EPA, 2002a), it was assumed that at lower levels of exposure typically encountered environmentally, no chemical interactions are expected (i.e., simple dose/concentration additivity would apply). For additivity to hold true, a further assumption must be that all the common mechanism chemicals exhibit similar pharmacokinetic and pharmacodynamic characteristics (i.e., distribute to the same target tissue and have similar elimination half-lives; exert toxicity via similar biochemical mechanisms, without specific regard to which organ is the critical target). In reality though, a case study of cumulative risk assessment of 33 organophosphorus pesticides provided BMDL (lower bound benchmark dose at ED_{10}) with a range of 3977-fold (female) to 5528-fold (male) difference between the highest BMDL (for malathion) to the lowest BMDL for (dicrotophos) (U.S. EPA, 2002b; Table I.B.-4). These 3-4 order of magnitude differences among “common mechanism chemicals” suggest that the PK and PD may each be quantitatively different. For example, if each compound is active through its oxon form, and each has a similar potency, then one may expect a 3-4 order of magnitude difference in PK, which seems unlikely. If the PK of these two compounds are similar, then potency would have to differ some 3-4 orders of magnitude. Inasmuch as neither potency nor PK may be envisioned to differ to that degree between these

chemicals, it seems logical to assume that both PK and PD differ between these chemicals. Differences in the extent of tissue exposure to toxicologically active chemical moieties with markedly difference potencies can be anticipated. These toxicological interactions are discussed a bit further in a hypothetical example in the following paragraph. Thus, the probability of toxicological interactions at the level of PK and PD exists.

- Are these toxicological interactions likely to cause higher toxicity (i.e., synergism or potentiation) or lower toxicity (i.e., antagonism or inhibition) than the simple addition of the toxicity (dose addition or response addition) from all components of the mixture?

With the OP-pesticide mixture, synergism/potentiation or antagonism/inhibition are likely depending on the rates of pharmacokinetic or pharmacodynamic interactions. For instance, all six OP compounds are phosphorothioates (i.e., P=S compounds); they require activation to their respective oxon (i.e., P=O) compounds to become potent AChE inhibitors. This requires oxidative desulfuration mediated by cytochrome P450. Depending on the substrates (e.g., the parent OP insecticides) that can compete among themselves for P450-mediated oxidation (bioactivation) and the involvement of other metabolic processes (e.g., competing enzymes that may conjugate the parent chemical with glutathione, a detoxication reaction), more or less potent oxon compounds may emerge (the oxon of one insecticide may be more potent than the oxon of another pesticide in the mixture) as the predominant (most prevalent) metabolite. If available, information on this competition between parent chemicals for oxidation and competition between different enzymes for bioactivation/detoxication will determine if synergism/potentiation or antagonism may result.

In addition to compound differences in activation and detoxication, differences in the biological fate of the inhibition complex (Oxon-AChE) exist. Regardless of the OP examined, once the oxon form becomes bound to the AChE enzyme, that enzyme molecule is no longer capable of metabolizing acetylcholine. Once bound, other events occur, leading to either destabilization and ultimate dissociation of the inhibitory complex or stabilization and aging of the inhibitory complex. Each of the OP compounds identified is an effective inhibitor, but they differ in the half-life of the inhibitory complex with some dissociating within hours whereas others may take days. Once bound, the inhibitory complex may “age,” and become permanent. Once aging occurs, the enzyme cannot be reactivated and AChE activity can only be restored with

the generation of new enzyme. Each of these OP compounds have different inhibition half lives and they differ in the extent to which they age, particularly so for dimethyl versus diethyl substituted compounds. When encountered in a mixture, compounds which bind readily, but which have a short inhibition half-life and relatively reduced potential to age, may actually be protective against AChE inhibition produced by other OP compounds.

Similarly, depending on the competitive inhibition of cytochrome P450 2E1 in the initial metabolism of TCE by the other chlorinated hydrocarbon solvents/volatile organics, the GST conjugation pathway may be favored (Dobrev et al., 2002); this would lead to more production of reactive thiols for possible renal carcinogenesis—an example of interactive toxicity.

One of the greatest challenges in dealing empirically with chemical mixture toxicology is the exponential rise of experimental groups as the number of component chemicals and doses increase. Frequently, systematic experimental studies of chemical mixtures become untenable because of the limitation of resources. Here, the possible application of reaction network modeling[3] (Klein et al., 2002; Liao et al., 2002; Reisfeld and Yang, 2004; Yang et al., 2004a,b) can offer a workable solution to the complexity of chemical mixture toxicology, particularly relating to metabolism.

- Under the exposure conditions and levels, are these toxicological interactions possible?

In the cases of pesticide applicators, total exposure comprises both occupational exposures and additional exposures from drinking water, foods and household residues. For these individuals, total exposures may approximate the range where toxicological interactions are possible (for Mixture 1). Similarly, occupational exposures in chemical industry or manufacturing plants where TCE, PERC, MC and CHF are used, plus additional exposures from drinking water, foods, showers, etc. could also render the cumulative exposure concentrations to where toxicological interactions are likely.

2.2.5. Step 5. Identify Chemicals in the Candidate Cumulative Assessment Group (CAG) for Further Evaluation. Select chemicals, chemical uses, routes and pathways

[3] Reaction Network Modeling: A chemical/petroleum engineering computer simulation technology to model the entire oil refinery based on initial chemical analyses of the feedstocks, as well as using graph theory, linear free energy relationship (LFER), computational quantum chemical calculations, quantitative structural activity correlation, Monte Carlo and quardrature modeling techniques. It is capable of simulating the fate of thousands of chemicals and tens of thousands of reactions simultaneously and predicting the outcomes of these chemical reactions.

from the CMG that have an exposure and hazard potential to result in cumulative effects (joint toxicity) for inclusion in the quantitative estimates of cumulative risk.

This determination flows from the information acquired during Steps 1-4 and compiled in Step 4. For the purpose of an illustration for the present project, we will assume that all the component chemicals in the respective Mixtures 1 and 2 are the respective CAGs. In the event that (1) exposures are sufficiently small or (2) exposure or toxicity data are too limited to support including a chemical in the CAG, it will be omitted at this stage. Once again, depending on the importance of the mixture of interest to public health, this (the omission) may not be acceptable. In that case, there are two alternatives: (1) There are insufficient data to conduct cumulative risk assessment and the component chemicals in the mixture should revert back to the traditional single chemical risk assessment; or (2) New research initiatives are to be launched to fill the data gaps for the specific purpose of conducting dosimetry-based cumulative risk assessment for the mixture of interest.

2.3. PHASE II: DOSIMETRY-BASED CUMULATIVE RISK ASSESSMENT

For those chemical mixtures selected to undergo DBCRA, the following additional five steps should be carried out. It is proposed that starting from Step 6 (i.e., the first step in Phase II), PBPK modeling should be incorporated. Common belief is that PBPK modeling is resource-intensive and it is difficult, particularly for models involving chemical interactions at the level of either pharmacokinetics or pharmacodynamics. This warrants some special discussion: First, all of the component chemicals in the mixtures used as examples in this document are industrially or environmentally important chemicals with uses and/or exposures of concern. When many chemicals reach this stage of commercialization, a substantial number of studies may have been conducted on them. Those chemicals have already become resource-intensive during the developmental stage to become successful chemicals in commerce. However, this is not the case for all commercially important chemicals. The important point is that quantitative, time-course data useful for PBPK model development may have already been generated during the product developmental phase. If the incentive (i.e., risk assessment-driven scientific studies) exists, such quantitative, time-course data would be generated during the product developmental phase automatically. In fact, PBPK modeling, being a hypothesis-testing tool in toxicology, may be utilized to conduct many different kinds of experiments on computers (i.e., *in silico* toxicology). Development of *in silico* toxicology such as PBPK modeling and other biologically based computer modeling will improve the “attrition rate” of drug

or chemical product development. Thus, it may save precious resources by avoiding unnecessary experimental studies or minimizing animal experimentation. Second, while PBPK modeling is by no means a very easy technology, it is not any more difficult than some of the statistical modeling (e.g., linearized multistage carcinogenesis model) carried out in the routine risk assessment process. Furthermore, excellent training opportunities are available and the development of software is such that more and more user-friendly tools are going to be available.

The advantages of incorporating PBPK modeling in CRA are many: First, PBPK modeling may bring the tissue dose, instead of the applied dose, into dose-response assessment. Thus, it is much more accurate in that pharmacokinetics has been employed to refine "exposure" in terms of tissue doses, reducing uncertainty. Second, PBPK modeling has the capability of incorporating toxicological interactions of multiple chemicals, representing a valuable feature for CRA. Third, PBPK modeling has the capability of extrapolation, be it dose-, species-, route-, age-, gender-dependent extrapolations. In many cases, such extrapolation may reach the region (e.g., very low doses) where experimental studies are impossible to conduct. Finally, PBPK modeling, in conjunction with Monte Carlo simulation, may estimate the true means (i.e., of tissue AUC values) by carrying out numerous repeated exposure simulations via computer-based programs. However, confidence in mean values will be tempered by the degree to which the distributions of the variable input parameters (i.e., chemical specific values such as partition coefficients and species specific values such as organ blood flows) are characterized.

2.3.1. Step 6. Characterize Dose-Response, Point of Departure and Determine Relative Potency from Tissue Doses. The risk assessor should select and apply an appropriate method to characterize the dose-response relationship for effects and determine the relative toxic potencies of the CAG chemicals by each exposure route and duration of interest. Subsequently, the point(s) of departure for extrapolating the risk of the CAG should be chosen. As indicated in the guidance document (U.S. EPA, 2002a), the preferred point of departure is one derived from modeling the dose-response curve of the index chemical to derive a BMD that estimates a pre-specified level of response (e.g., BMD_{10}, BMD_5 or BMD_1). The utility of PBPK modeling in the dose-response analyses is to provide tissue dose metrics such that in the BMD estimation, the tissue doses rather than the applied doses will be used in the Benchmark Dose Software (BMDS). Obviously, this requires identification of the target tissue. If a PBPK model capable of addressing chemical interactions (be they additive or not) cannot be

constructed because of data gaps, PBPK models for individual chemicals (e.g., the OP pesticides) should be constructed to determine tissue dose metrics (e.g., concentration of active metabolite in brain tissue) based on PBPK modeling of given applied doses. That is, given the exposure level (applied doses), what would be level of the dose metric in the affected tissue (e.g., brain) relative to the level of inhibition of brain AChE (e.g., a 10% reduction in AChE activity) and the related clinical signs? Pharmacokinetic models can simulate concentrations of toxicants in tissues for which biological monitoring cannot be conducted in humans and for which concentration data may not be available in test animals. Certainty in PBPK-based risk assessments is increased when there are tissue toxicant concentration data against which to compare PBPK model predictions. The availability of tissue toxicant concentration data for comparison should be considered when choosing a pharmacokinetic outcome (tissue concentration, dose metric) for risk assessment application. Of course, preferably, an interactive PBPK model is available at this point such that pharmacokinetic and pharmacodynamic interactions may be taken into consideration and "interaction thresholds" (El-Masri et al., 1996b; Dobrev et al., 2001, 2002) may be estimated to obtain interactive NOAELs, LOAELs (which may be designated as $NOAEL_{Int}$ and $LOAEL_{Int}$) and higher dose-response levels.

When dose additivity is selected as the cumulative risk assessment model and once the models have been constructed, the index chemical should be selected and the potencies of other chemicals in the CMG should be determined based on dose response relationships characterized at the level of the dose metric most associated with the response. This might, for example, be the level of the dose metric corresponding to the animal ED_{10}. Specifically, the dose-response relationships should be developed on the basis of internal, rather than external dose. In this manner, pharmacokinetic interactions that alter the dose metrics of interest can be made valuable in estimating the response from the mixture.

2.3.1.1. Recommendations on Modeling — The U.S. EPA guidance document included an informative discussion on "Modeling the Data" (Section 6.2.1.6, U.S. EPA, 2002a). Although it is not necessarily for PBPK modeling, the discussion is relevant to the present effort. Some passages are quoted below and the CRA risk assessors using PBPK modeling should abide by these recommendations as much as possible:

> …The selection of a mathematical model structure to fit the data being analyzed should be guided by the biology of the common mechanism of toxicity, the toxicokinetics of the chemicals, and the observed shapes of their dose-response curves and the experimental designs used to

generate the data. If available, pharmacodynamic and pharmacokinetic data should be considered in order to account for tissue concentrations and to aid in defining dose-response relationships across different species, routes and time-frames of exposure…

…Although it is not possible to recommend the use of specific models, a few points that should be considered in modeling the data follow:

- Modeling of individual animal data is desirable; however, if this is not practical, then use of summary data such as means and standard deviations can be alternatives
- Care should be taken with modeling high-dose data (particularly extreme doses) because the model shape in the low-dose region can be influenced by high-dose data
- Log transformation of data should be justified because such a transformation may distort the dose-response curve
- Data variability should be described by appropriate statistical techniques and reflected in the potency estimate (e.g., by weighting the data in the fitting procedure)
- Confidence intervals or limits should be included in the analysis because they can be valuable for evaluating the influence of variability on the potency estimates
- An estimate for the uncertainty of the model used in the analysis should be included
- The statistical fitting method used must be clearly described.

2.3.1.2. Interactive PBPK Model: General Information — As this is the first step of the dosimetry-based cumulative risk assessment process and the step where PBPK modeling initiates, it is appropriate to discuss the issues related to the incorporation of PBPK modeling. In addition to the discussion below, a recent paper on PBPK/PD modeling is attached as Appendix A.

From the perspective of interactive PBPK modeling, two aspects need to be addressed: pharmacokinetic interactions and pharmacodynamic interactions. As indicated earlier under Step 4, pharmacokinetic and pharmacodynamic interactions are likely in both Mixtures 1 and 2. Thus, it is necessary to construct interactive PBPK models after a thorough review of the existing literature. The most ideal and scientifically defensible data requirement for establishing an interactive PBPK model is that each component chemical in the mixture already has its respectively established PBPK model and that there are many pharmacokinetic datasets in laboratory animals

as well as in humans available for each of these component chemicals. In some cases, even more specific and stringent data requirements are needed; an example may be the ultralow dose pharmacokinetic data in perinatal developmental stages of laboratory animals for extrapolation to human fetuses, neonates and infants using PBPK modeling. However, until such time that quantitative time-course data useful for PBPK modeling are automatically part of the product developmental process, the most likely scenario is imperfect datasets. In Mixture 1, no PBPK models for fenthion, fenitrothion, diazinon, and methyl parathion could be located at this time. However, PBPK models do exist for parathion (Sultatos, 1990; Gearhart et al., 1994; Abbas and Hayton, 1997; Gentry et al., 2002) and chlorpyrifos (Timchalk et al., 2002a,b; Kousba et al., 2003). In Mixture 2, because all the chemicals are important solvents with huge production volumes, PBPK models are available for TCE, PERC, MC and CHF. TCE has obtained perhaps the most attention of these chlorinated hydrocarbons/volatile organics in terms of the development of PBPK models, many of which have also been used to support risk assessment (Sato et al., 1977, 1991; Bogen, 1988; Koizumi, 1989; Fisher, 1993; Fisher and Allen, 1993; Fisher et al., 1989, 1990, 1991; Allen and Fisher, 1993; Clewell et al., 1995; Cronin et al., 1995; Poet et al., 2000; Dobrev et al., 2001, 2002). Several PBPK models for the disposition of tetrachloroethylene were presented for animals and/or humans (Ward et al., 1988; Koizumi, 1989; Bois et al., 1990; Gearhart et al., 1993; Rao and Brown, 1993; Byczkowski et al., 1994; Dallas et al., 1994, 1995; Wilson and Knack, 1994; Byczkowski and Fisher, 1995; Reitz et al., 1996; Loizou, 2001; Poet et al., 2002).[4] PBPK models for MC alone or for mixtures of MC and TCE or other chlorinated solvents have been published (Reitz et al., 1988; Koizumi, 1989; Tardif and Charest-Tardif, 1999; Dobrev et al., 2001). Several PBPK models that included progressively more sophisticated levels of biochemical complexity (e.g., relating to metabolite formation, cellular regeneration, enzyme inhibition) have been developed for chloroform (Corley et al., 1990, 2000; Gearhart et al., 1993; Chinery and Gleason, 1993; McKone, 1993; Roy et al., 1996; Levesque et al., 2000).

2.3.1.3. PBPK/PD Models: Data Needs — What are the specific data needed for building PBPK models? And what happens when such data are missing because no

[4] PBPK models developed for a single chemical may differ for many reasons, including the species addressed, route of exposure simulated, effect evaluated, choice for dose metric developed, differential reliance on *in vivo* datasets and/or *in vitro* datasets, choices for the values of physiological and chemical-specific parameter values employed, etc.

PBPK modeling has been attempted on some of the components in the mixtures of interest? First, the data necessary for establishing a PBPK model for a single chemical will be presented. Obviously, well conducted *in vivo* pharmacokinetic experiments are essential and usually the more varied the datasets (e.g., different doses, routes, species), the better. Other PBPK modeling-specific information such as the chemical-specific parameters (e.g., tissue partition coefficients, V_{max}s and K_ms) is needed. Enzyme kinetic data, particularly human data, of the bioactivation (e.g., P=S to P=O conversion) and detoxication (e.g., oxidative cleavage, hydrolysis, oxidative dealkylation, etc.) processes will be important for the interactive PBPK model. *In vitro* determination of tissue partition coefficients and enzyme kinetic data are relatively straightforward. A later section is devoted totally to high quality organ donor liver enzyme studies. Alternatively, with modern genetic engineering technologies, many human enzymes are available commercially, though their expression, singly, in non-mammalian species may complicate the extrapolation of results obtained from such preparations. For instance, several recombinant human CYP enzymes are now commercially available, including CYP 1A1, CYP 1A2, CYP 2B6, CYP 2C8, CYP 2C9, CYP 2C18, CYP 2C19, CYP 2D6, CYP 3A4, CYP 3A5, and CYP 3A7 (e.g., from GenTest [Woburn, MA], Cypex Ltd. [Dundee, Scotland], and Sigma-Aldrich [St. Louis, MO]). Thus, heretofore unavailable human enzyme kinetic information for many of the environmentally important chemicals is within easy reach for many laboratories. These experiments should be performed.

Pharmacodynamically, for instance, once the oxon (i.e., P=O) derivatives are formed, a number of questions arise: What is the competition between the irreversible binding of the oxon derivative with red cell AChE, non-specific esterases, and brain AChE for any given single pesticide? The same question, although much more complex, should be asked about the mixture of 6 oxon derivatives. Here the ultra-low dose pharmacokinetic/pharmacodynamic studies in the binding of brain AChE with any given OP pesticide in the presence of others similar to those reported by Vogel et al. (2002) will be critical for the interactive PBPD modeling in the cumulative risk assessment. Both chemical specific and species specific data are required. Chemical specific data include metabolic rate constants and measures of solubility (e.g., partition coefficients). Species specific data include information on body weight, organ or compartment sizes, region/organ blood flow rates and media specific intake rates (i.e., alveolar ventilation rates). Body weight and relative contributions of specific organs (i.e., liver) to fractional body mass are fairly constant; they are usually modeled as point values. Regional or organ specific blood flows may vary, obviously with exercise;

recent evidence suggests that hepatic blood flow may vary three-fold at rest among adults. Virtually all species specific data may vary, to some extent, among individuals and with age, as may metabolic rate constants. Chemical-specific partition coefficients may vary approximately two-fold among individuals, and metabolic parameter values may vary markedly, depending on the chemical and enzyme involved.

2.3.1.4. PBPK/PD Modeling: Model Structures — While the structure of the PBPK/PD model will have to be determined in detail based on the analysis conducted in Steps 1-5, a general suggestion on what the model might contain can be provided at this time. Figure 4 presents a graphical representation of the PBPK model that could be initially considered. The model consists of several familiar compartments. The CNS/PNS (central nervous system/peripheral nervous system) compartment should be added and indeed, can be split into two compartments if kinetic processes are significantly different in the two and if endpoints or validation datasets suggest doing so. The liver compartment is where metabolism is usually regarded as occurring, but extrahepatic metabolism should also be considered. For the drinking water scenario (or other ingestion scenarios) chemical exposure of the liver should be primarily via the portal flow from the GI compartment in addition to systemic flow. Portal blood flow may play a less prominent role (compared to arterial flow) for substances encountered via inhalation. Usually, the chemical is dosed directly to the GI compartment and first order uptake is often assumed, but more complex formulations can be used if modeling parsimony is not overly compromised.

In the diagram, the liver compartment includes a schematic of simplified metabolic pathways. For metabolism, parsimony is important: only include discrete pathways if the data will support developing kinetic parameter estimates. The overall metabolism can be simplified in a number of ways: sequential metabolic steps can be modeled as one step that represents the rate-limiting step in the sequence. Parallel pathways can be lumped together. Insignificant pathways can be ignored. The extent to which a given pathway may be deemed insignificant should be judged on the basis of all information known for each component of the chemical mixture; a pathway deemed minor or insignificant for one chemical may be critical for another. Because the same model structure should be employed for each chemical (within reason) and for the mixture, the selection of pathways for inclusion should not be taken lightly. Some pathways can be multiple steps (e.g., oxidative desulfuration and hydrolysis) while others can be single staged (e.g., clearance pathways to non-toxic non-interacting metabolites). The importance of parsimony is emphasized by the limited amount of

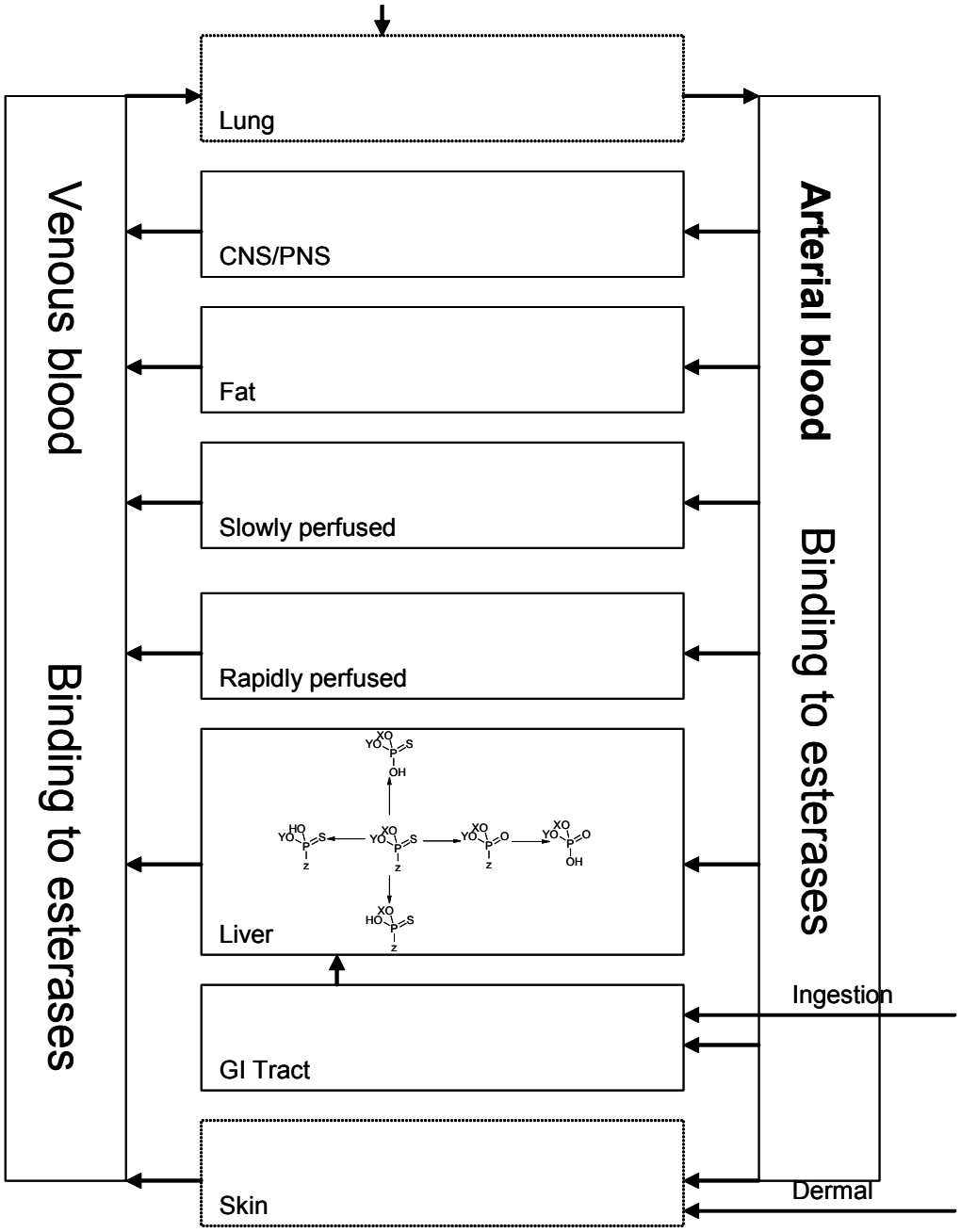


FIGURE 4
A Preliminary PBPK Model Structure for OPs

data (particularly *in vivo*) and the difficulty of extrapolating *in vitro* kinetic constants to *in vivo* or determining the constants from model optimization. Automated model optimization of parameters should be minimized because, for the single chemical models, each pathway can require 2 metabolic constants, there can be 2, 3, 4… different metabolic steps, some of these steps could actually inhibit each other if the same enzymes are used at high enough doses, and inhibition would require additional parameters. So for metabolism alone, it would be easy to get over 10 unknown parameters, which would be difficult to optimize with any certainty. This is before consideration of inter-component PK interactions, which require yet more optimization. However, more than one metabolic step will nevertheless be required.

In the schematic (Figure 4), lung and skin compartments are dotted boxes to represent the fact that they may or may not be required for the drinking water assessment. If dermal exposure to drinking water sources does not contribute significantly (a judgment call) to the aggregate exposures, the dermal compartment may be unnecessary for the initial model, but may be desirable for future modeling work (especially for occupational exposure PBPK models). Depending on the chemical and its volatile properties, only occupational exposure models may require the inhalation route of exposure. As with the discussion of age and gender, the model is usually built for the route where the most complete datasets are available and then extended to other routes. However, for this study, the ingestion route should be given decided preference unless data are severely lacking. If inhalation models are developed, attention must be paid to the form of the chemical (i.e., aerosols) and non-equilibrium processes in uptake kinetics.

The CNS/PNS compartment will need to include a submodel for binding to AChE. A simple conceptualization of this is proposed in Figure 5. As OP binding is considered largely irreversible and causes complete inhibition of the enzyme (individual enzymes that are bound), the degree of inhibition would reflect the number of bound enzymes. However, the activity of AChE is not necessarily proportional to unbound (free) enzyme; there may be a “reserve capacity” in that not all enzyme is required to clear acetylcholine in synapses. Thus, the response may be a non-linear function of the molecular events. PD models will be a useful way to describe these processes once developed.

The PD submodel should include a description of the process of synthesis of AChE, an on-going process. The kinetics of this process may be zero order (as is often assumed for glutathione synthesis (D’Souza and Andersen, 1988), or could be inducible. Binding of free AChE by activated OPs may be a saturable process

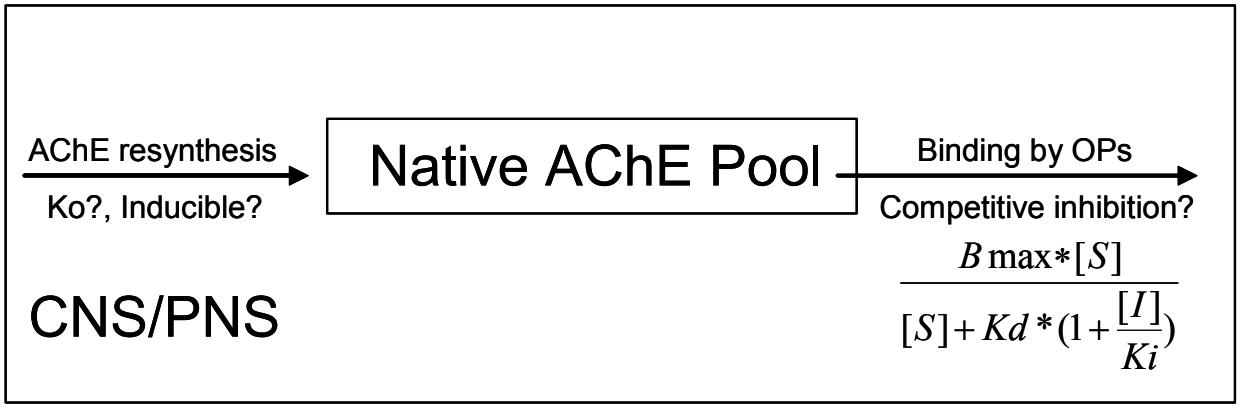


FIGURE 5
A Pharmacodynamic Submodel for CNS/PNS Compartment

describable by a Bmax or Hill's equation [Bmax + S/(S+Kd)] with or without inhibition by competing OPs. However, since it is usually regarded as irreversible, a linear equation may do equally well and will involve fewer parameters. An empirical relationship between the concentration of free AChE and neurological effects (e.g., tremors) will complete the PD portion of the model. Similar submodels will need to be included for binding to other esterases, primarily in the blood.

In order to attain reasonable level of confidence, the interactive PBPK models for mixtures should be developed only after the component single chemical models are developed and validated. Likewise, the PD submodels should not be incorporated until the PBPK models are as validated as possible.

The models can be exercised with existing animal data to determine practical thresholds for toxicokinetic interactions. In the event that human exposure is at lower ranges than the model is validated for, particular attention should be paid to the presence or absence of interactions at the lowest validated level. It would be useful to perform low-dose PK studies in animals (or humans) to extend the range of the model downwards, especially if it can be extended down to the range of the exposures under consideration. Moreover, it would be even more important to do the low dose experiments if (non-additive) interactions are still occurring at levels below the model validation range, to determine the shape of the dose response curve below the point of departure.

2.3.1.5. Human PBPK Modeling: Incorporation of *In Vitro* Enzyme Studies —

The ultimate goal of PBPK modeling is to provide scientifically defensible computer simulations of the fate of the chemical or chemical mixtures in humans. There are ethical and other problems with *in vivo* human toxicology studies, particularly with highly toxic pesticidal chemicals such as OPs. Thus, the building of human PBPK models must rely on allometric extrapolation of animal data and/or *in vitro* studies using human tissues. While many of the physiological parameters on humans are readily available in the physiology literature, some parameters which are chemical specific such as tissue/blood partition coefficients and metabolic parameters (K_m and V_{max}) are best empirically derived. Recent advances in PBPK modeling have demonstrated the ability of the technique to include extrapolations made from data on chemical metabolism and enzyme contents derived from *in vitro* human and research animal tissue preparations (Kedderis and Held, 1996; Kedderis, 1997; Lipscomb et al., 1997, 1998, 2003a,b; Lipscomb and Garrett, 1998; Snawder and Lipscomb, 2000; Kedderis and Lipscomb, 2001; Lipscomb and Kedderis, 2002). In particular, careful experimental measurements

and statistical evaluations on the content of microsomal protein, cytochrome P450 enzymes, in human liver samples derived under organ transplant conditions had been carried out (Lipscomb et al., 1997, 1998, 2003a,b; Lipscomb and Garrett, 1998; Snawder and Lipscomb, 2000). This is important because some past human studies employed liver samples from cadavers, which were metabolically compromised. Lipscomb et al. (2003b) recently integrated their various human *in vitro* studies from organ donors and demonstrated the application of such information via PBPK modeling in a risk assessment framework. This paper (Lipscomb et al., 2003b) provided a stepwise illustration of how to incorporate three different datasets (the microsomal protein content of human liver, the CYP2E1 [the principal metabolic enzyme for TCE] content of human liver microsomal protein, and the *in vitro* V_{max} for TCE oxidation by humans) into a PBPK model for risk-relevant pharmacokinetic outcome in humans. Using a variety of statistical analyses, the 5^{th} and 95^{th} percentiles of the resulting distribution on V_{max} (TCE oxidized per minute per gram liver) differed by approximately 6-fold. These values were converted to the *in vivo* V_{max} (mg TCE oxidized/hr/kg body weight) and incorporated into a human PBPK model for TCE. Model simulation under the conditions of 8-hr inhalation exposure to 50 ppm or drinking water exposure at 5 μg TCE in 2 L/day revealed that 6-fold variation in V_{max} (i.e., V_{max} at 5^{th} or 95^{th} percentiles levels) resulted in only 2% or less differences in metabolism of a key intoxicating step, the formation of chloral hydrate (Lipscomb et al., 2003b). On the surface, this finding, which is suggestive of V_{max} insensitivity under the model simulation conditions, has strong implications in risk assessment. In essence, it suggests that, at low environmental or occupational exposure conditions, individual variability of metabolic capacity as large as 600% (6-fold) has little or no impact on the toxic outcome of TCE. However, upon closer examination, valid scientific explanation is available. Kedderis (1997), in studying the effect of enzyme induction on the bioactivation of TCE and other volatile organic compounds, indicated that the hepatic blood flow limitation plays an important role in the kinetics of bioactivation. What happens in the above situation (Lipscomb et al., 2003b) is that, even though the individual metabolic capacity varies greatly (i.e., 600%), the rate of hepatic blood flow delivery of TCE and its related metabolites (formed from earlier passages through liver cells) to the liver is much slower than the rate of bioactivation in the liver. Thus, this “flow-limited process” (that delivers concentrations below those that are in the linear range of the metabolic rate versus substrate concentration curve) is a more important factor than maximum metabolic rate, thus limiting the impact of the large variability in metabolic capacity in the population. These results underscore the importance of considering the overall dynamic equilibrium

of all the relevant biological processes in the body—in some sense, a broader application of the systems biology approach. The Lipscomb et al. (2003b) paper is attached as Appendix B as instructional material for the incorporation of human *in vitro* data into the PBPK modeling process.

Appendix C provides summary information for each of the component chemicals in the two mixtures identified for this project. It includes information on the toxicity, metabolism and pharmacokinetics for each chemical.

2.3.2. Step 7. Develop Detailed Exposure Scenarios for All Routes and Durations. The contribution to isolated or individual chemical exposures to the potential magnitude of exposure should be characterized. Once these are characterized, a decision should be reached as to whether these exposure scenarios should be included in a qualitative exposure assessment. Consideration should be given to the identification of subpopulations and their locations, as well as to co-exposures to multiple chemicals. As drinking water is the primary concern for this project, emphasis will be placed on the oral consumption of finished drinking water. Review of pertinent literature on the component chemicals in the mixture is the first step (see Appendix C). In the guidance document (U.S. EPA, 2002a), it was stressed that cumulative risk assessments should reflect use patterns and practices on a scale sufficient to capture the variability in pesticide use, but not so large as to inappropriately dilute real and significant differences. A specific example was given on fenthion, one of the six OP pesticides selected by the U.S. EPA for the first mixture. Apparently, fenthion was used for localized mosquito control in parts of southern Florida; therefore, it was stated that this pesticide should have only limited consideration in an assessment of other OP pesticides, including those used for mosquito control (U.S. EPA, 2002a). Exposure assessments should take into account those factors that impact the targeted group of humans or the geographic region of interest.

2.3.3. Step 8. Quantify Parameters for Exposure. Exposure should be presented in as quantitative a manner as is possible. The magnitude, frequency and duration for all pertinent exposure pathway/route combinations should be determined. Appropriate sources of use/usage information, chemical concentrations in all appropriate media, and any modifying factors necessary should be included in the assessment. Where necessary, surrogate datasets developed for similar chemicals, published literature or generic datasets should be identified and justified for inclusion. Here, emphasis will be placed primarily on drinking water. The guidance document (U.S. EPA, 2002a)

indicated that evidence of the co-occurrence of pesticides within a drinking water source for a CAG is a critical piece of information needed prior to making a decision to include more than one pesticide in a cumulative drinking water exposure assessment. Direct measurements of combinations of pesticides in finished drinking water are rarely available. However, U.S. Geological Survey's National Water Quality Assessment Program databases do contain information on the co-occurrence of a wide variety of pesticides in ambient surface water, and some registrant-sponsored studies provide co-occurrence data for some compounds in drinking water. The exposure scenarios may take on one of several forms, as previously described. In accordance with U.S. EPA guidance (2002a), the exposure data will be formatted for input into the quantitative DBCRA taking into account, as appropriate, seasonal variations, geographical variations, and other variations in exposure. The range of exposures determined as such must be part of the exposure input. Yet more sophisticated approaches, such as the one suggested by Figure 6, can also be considered. In Figure 6, the distributions of exposures are determined and used as input to a PBPK model. As interactions between component chemicals in the CAG will depend on the magnitude and duration of the exposure to each chemical, non-PBPK models would necessarily have to attempt to simplify the basis for the interactions in some empirical manner. Alternately, PBPK models (should validated ones be developed) can be used to estimate the actual extent of toxicity (AChE inhibition or as otherwise desired). The model can be run iteratively for as many exposure scenarios as necessary. Indeed, it is quite feasible to use a Monte Carlo sampling approach to sample from the distributions. For example, within each of 500 communities, the simulation of 1000 exposures (i.e., what 1000 persons would be exposed to) would not be an unreasonable task. This would allow the determination of cumulative risks in various locations, times of year, etc., as well as for the nation as a whole.

2.3.4. Step 9. Conduct Dosimetry-Based Cumulative Risk Assessment. This is the point in the process where the pharmacokinetic data and model are combined with the defined exposure scenario(s) to estimate internal dosimetry. The resulting internal doses are combined with data describing dose-response and potency defined in step 6. To accomplish this, route/duration-specific dose metrics associated with specific risks should be identified and internal doses should be expressed in these terms. A trial run should be conducted initially and its results evaluated. The model should be subjected to a sensitivity analysis, which will identify model assumptions and parameters that most influence the production of the risk related dose metric. An assessment of the

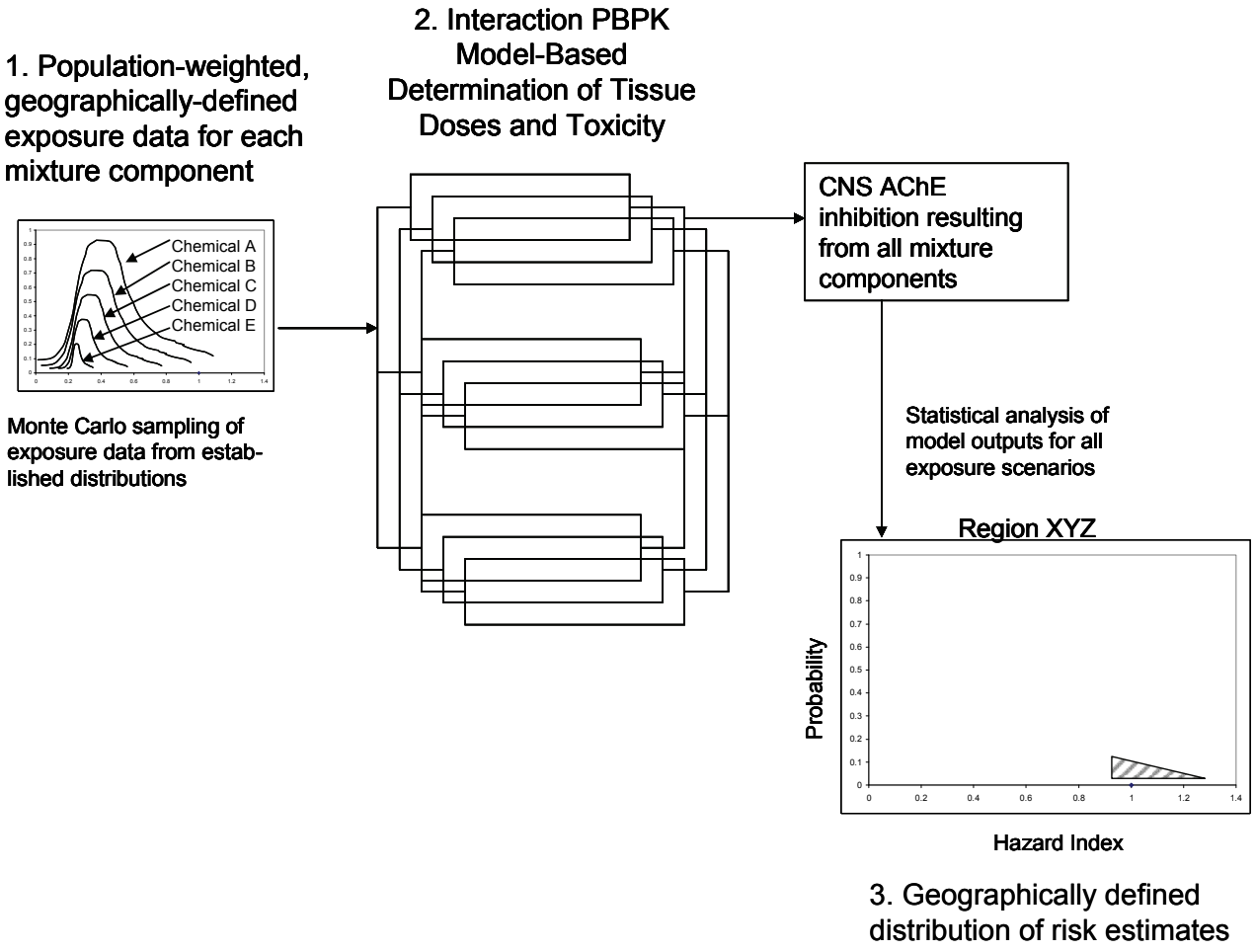


FIGURE 6

A Schematic for Integrating Data for Cumulative Risk Assessment. This is a possible method for integrating exposure distributions with PBPK models for several interacting chemicals and analyzing model output to determine distribution of cumulative risk estimates.

subpopulations of concern should be undertaken, and uncertainty and FQPA safety factors should be recommended. The documents *Cumulative Risk: A Case Study of the Estimation of Risk from 24 Organophosphate Pesticides* (U.S. EPA, 2000b) and *Organophosphate Pesticides: Revised OP Cumulative Risk Assessment* (U.S. EPA, 2002b), as well as the documents addressing the cumulative risk of N-methyl carbamates, triazines and the chloroacetanilides should be consulted for carrying out cumulative risk assessment. The results of the DBCRA should be presented so that the reader will understand which dose metrics for which chemicals and effects were selected and simulated, and the impact that a cumulative exposure will have on the production of these dose metrics and, ultimately, on risk.

Assigning FQPA Safety Factors is restricted to children and restricted to pesticide chemicals only (refer to OPP guidance and to Step 10, below).

2.3.5. Step 10. Characterize Cumulative Risk via Pharmacokinetic Analysis. Describe the results and conclusions of the cumulative risk analysis, including the relative confidence in toxicity and exposure data sources and model inputs. Discuss major areas of uncertainty, the magnitude and direction of likely bias, and the impact on the final assessment. Evaluate the risk contributions from each pathway and route individually, as well as in combination. Identify risk contributors with regard to chemical(s), pathway, source, time of year, and impacted subpopulation (with particular attention to children). Conduct sensitivity analyses to determine those factors most likely to impact the risk. Determine need for additional uncertainty and safety factors.

A summary of the risk characterization should include a restatement of the scope of the issue being addressed, explaining the chemicals under evaluation, data available and their strengths and weaknesses including uncertainties, the assumptions made during the analysis, and how the results are interpreted for each of the demographic groups represented. These data include those pertaining to the temporal and geographic nature of exposure, including food and water ingestion rates, contamination levels (including non-detects) and the methods used to develop distributions for variable data. Special emphasis should be placed on how groups differ, and the likely bases for those differences, including anatomic, biochemical and physiologic differences, as well as differences related to exposures. With respect to PBPK modeling, these differences may be due to age and sex-dependent differences in body composition and/or metabolic capacity. The risk characterization section should report biases in datasets employed, and a general evaluation of the level of confidence placed in the analysis. Significant sources of uncertainty should be communicated, and when possible, the

results of a sensitivity analysis should be used to demonstrate the level of impact that these uncertainties may have on the overall outcome. Outcome should be communicated as the level of the dose metric(s) most closely associated with the health endpoints of concern. Regarding sensitivity within the human population, the application of FQPA safety factors is restricted to children and to pesticide chemicals. The safety factor and the bases for its derivation (a value of up to a factor of ten) have been described in other documents developed by OPP. When data and circumstances warrant, the additional FQPA safety factor may not be applied. When applied, however, the factor is applied to the results for the chemical mixture, rather than to the individual chemicals.

3. CONCLUSIONS

In this report, we outline some of the considerations that are involved in performing CRA using a PBPK-modeling based approach. While this report makes a number of specific suggestions and observations, additional details of a PBPK-based approach to CRA will depend on the specific chemicals in a CAG, which are not known until the initial analysis is complete; and issues that arise during the risk assessment process itself, which are largely unknown until the work is undertaken. For methodological issues that are decided on the basis of scientific judgment, decisions should be made with the assistance of appropriate advisory committees dedicated to the subject at hand.

PBPK modeling-based approaches offer several advantages in CRA. First, U.S. EPA has recently advocated using biologically-based approaches in risk assessment. Indeed, these biologically based approaches offer the best means of performing many of the extrapolations that are necessary in the risk assessment process. Biologically based approaches were further recommended by U.S. EPA for use in CRA, when they are available. Because of their ability to extrapolate dosimetry across dose, species, sex, route and age, PBPK modeling is often the favored biologically-based method for determining tissue dosimetry. Second, PBPK modeling is the simplest method for characterizing PK interactions in the body that is directly based on the biology of the process. Other methods are empirical and not only take a significant effort to develop, but have uncertainties when extrapolations are made to scenarios that are untested (e.g., empirical methods developed for A+B and B+C do not translate easily into a method for A+C). Third, if significant PK or PD interactions occur, DBCRA cannot be reasonably performed without addressing the interactions at the level of target tissue concentration. In other words, why go from single chemical risk assessments to multiple chemical risk assessments when one of the ramifications of multiple chemical exposures (toxicokinetic interactions resulting in altered tissue dosimetry) is omitted?

Implementing PBPK modeling-based approaches to CRA would require the development of PBPK models for the individual chemicals in the CAG as well as a quantitative definition of the nature of the interactions. While PBPK models exist for some of the chemicals considered here, some data are available to support the development of PBPK models for the rest. Most likely, the biggest data gap consists of data regarding interactions. For the chlorinated solvents, sufficient data may be available at this time; indeed some interaction models are already in the literature (Dobrev et al., 2001, 2002; Haddad et al., 2000; Thrall and Poet, 2000). For the OP

pesticides, data gaps for some combinations of chemicals are likely. Nevertheless, the burden of developing PBPK models for this group is greatly reduced by the fact that the same model structure can likely be used for all six chemicals. With sufficient data, building the interactive PBPK models is likely to be feasible.

For drinking water scenarios, multiple route (drinking, inhalation and dermal through bathing) exposure is a possibility. Even if the CRA is restricted to direct consumption of drinking water itself, the pharmacokinetic and pharmacodynamic processes involved may be non-linear, including differing rates of metabolism for different chemicals, inhibition and replenishment of AChE, etc. These types of non-linear processes are well characterized by PBPK models. Moreover, the drinking water scenario involves a complex set of exposures. For each chemical, a different distribution of exposures, varying by season of the year, spatial location, and other factors must be incorporated into the assessment. When this level of complexity is added to the variety of issues mentioned above, simple algebraic approaches to calculating risks become strained. Other modeling approaches may be better suited to address the exposure assessment part of the CRA, and these approaches are easily tied into the PBPK model.

The approach recommended in this report is completely consistent with existing U.S. EPA guidance. The PBPK model itself largely affects the dose-response analysis of the CRA. In essence, a PBPK modeling approach is one alternative method suggested by U.S. EPA in performing dose-response analysis. However, if a PBPK model is to be used, other aspects of the CRA are also affected. The approach used for exposure assessment, for example, must be tailored in such a way that the structure of the dataset is appropriate as an input to the PBPK model. As a second example, the criteria used to determine whether exposure to a chemical is sufficient to warrant its inclusion in the CAG is also modified by the fact that chemical interactions are addressed within the CRA. Specifically, this report points out that a chemical could conceivably be retained in the CAG based on an ability to interact with another chemical, regardless of whether the retained chemical can directly cause the toxicity that is the critical endpoint for the analysis. In this way, a PBPK modeling-based CRA may more accurately determine the actual cumulative risk from a set of chemicals than an approach that does not quantify the impact of interactions on toxicity. Last but not least, the ultimate goal for risk assessment is the protection of public health. Therefore, PBPK modeling of chemical and chemical mixtures in humans is absolutely essential. Accordingly, the incorporation of quality human tissue studies into the PBPK modeling process is of critical importance. As a final concluding remark, as U.S. EPA advances

the science of cumulative risk assessment, PBPK modeling should be incorporated where appropriate.

4. REFERENCES

Abbas, R. and W.L. Hayton. 1997. A physiologically based pharmacokinetic and pharmacodynamic model for paraoxon in rainbow trout. Toxicol. Appl. Pharmacol. 145(1):192-201.

Allen, B.C. and J.W. Fisher. 1993. Pharmacokinetic modeling of trichloroethylene and trichloroacetic acid in humans. Risk Anal. 13(1):71-86.

ATSDR (Agency for Toxic Substances and Disease Registry). 1995. Toxicological Profile for 1,1,1-Trichloroethane (CAS# 71-55-6). U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

ATSDR (Agency for Toxic Substances and Disease Registry). 1997a. Toxicological Profile for Trichloroethylene (TCE) (CAS# 79-01-6). U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

ATSDR (Agency for Toxic Substances and Disease Registry). 1997b. Toxicological Profile for Chloroform (CAS# 67-66-3). U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

ATSDR (Agency for Toxic Substances and Disease Registry). 1997c. Toxicological Profile for Tetrachloroethylene (PERC) (CAS# 127-18-4). U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

ATSDR (Agency for Toxic Substances and Disease Registry). 2004. Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA. Available at http://www.atsdr.cdc.gov/interactionprofiles/ipga.html.

ATSDR (Agency for Toxic Substances and Disease Registry). 2005. ATSDR Public Health Assessment Guidance Manual. U.S. Department of Health and Human Services, Atlanta, GA. Available at http://www.atsdr.cdc.gov/HAC/PHAManual/toc.html.

Bogen, K.T. 1988. Pharmacokinetics for regulatory risk analysis: The case of trichloroethylene. Regul. Toxicol. Pharmacol. 8(4):447-466.

Bois, F.Y., L. Zeise and T.N. Tozer. 1990. Precision and sensitivity of pharmacokinetic models for cancer risk assessment - tetrachloroethylene in mice, rats, and humans. Toxicol. Appl. Pharmacol. 102(2):300-315.

Bruckner, J.V. and D.A. Warren. 2001. Toxic effects of solvents and vapors. In: Casarett and Doull's Toxicology: The Basic Science of Poisons, C.D. Klaassen, Ed. McGraw-Hill, New York, NY. p. 892.

Byczkowski, J.Z. and J.W. Fisher. 1995. A computer-program linking physiologically-based pharmacokinetic model with cancer risk assessment for breast-fed infants. Comput. Meth. Programs Biomed. 46(2):155-163.

Byczkowski, J.Z., E.R. Kinkead, H.F. Leahy, G.M. Randall and J.W. Fisher. 1994. Computer-simulation of the lactational transfer of tetrachloroethylene in rats using a physiologically-based model. Toxicol. Appl. Pharmacol. 125(2):228-236.

Chambers, J.P., S.L. Hartgraves, M.R. Murphy, M.J. Wayner, N. Kumar and J.J. Valdes. 1991. Effects of three reputed carboxylesterase inhibitors upon rat serum esterase activity. Neurosci. Biobehav. Rev. 15:85-88.

Chanda, S.M. and C.N. Pope. 1996. Neurochemical and neurobehavioral effects of repeated gestational exposure to chlorpyrifos in maternal and developing rats. Pharmacol. Biochem. Behav. 53(4):771-776.

Chinery, R.L. and A.K. Gleason. 1993. A compartmental model for the prediction of breath concentration and absorbed dose of chloroform after exposure while showering. Risk Anal. 13:51-62.

Clewell, H.J., P.R. Gentry, J.M. Gearhart, B.C. Allen and M.E. Andersen. 1995. Considering pharmacokinetic and mechanistic information in cancer risk assessments for environmental contaminants: Examples with vinyl chloride and trichloroethylene. Chemosphere. 31(9):2561-2578.

Cohen, S.D. 1984. Mechanisms of toxicological interactions involving organophosphate insecticides. Fundam. Appl. Toxicol. 4:315-324.

Corley, R.A., A.L. Mendrala, F.A. Smith et al. 1990. Development of a physiologically based pharmacokinetic model for chloroform. Toxicol. Appl. Pharmacol. 103:512-527.

Corley, R.A., S.M. Gordon and L.A. Wallace. 2000. Physiologically based pharmacokinetic modeling of the temperature-dependent dermal absorption of chloroform by humans following bath water exposures. Toxicol. Sci. 53:13-23.

Cronin, W.J., E.J. Oswald, M.L. Shelley, J.W. Fisher and C.D. Flemming. 1995. A trichloroethylene risk assessment using a Monte Carlo analysis of parameter uncertainty in conjunction with physiologically-based pharmacokinetic modeling. Risk Anal. 15(5):555-565.

D'Souza, R.W. and M.E. Andersen. 1988. Physiologically based pharmacokinetic model for vinylidene chloride. Toxicol. Appl. Pharmacol. 95:230-240.

Dallas, C.E., X.M. Chen, S. Muralidhara, P. Varkonyi, R.L. Tackett and J.V. Bruckner. 1994. Use of tissue disposition data from rats and dogs to determine species-differences in input parameters for a physiological model for perchloroethylene. Environ. Res. 67:54-67.

Dallas, C.E., X.M. Chen, S. Muralidhara, P. Varkonyi, R.L. Tackett and J.V. Bruckner. 1995. Physiologically-based pharmacokinetic model useful in prediction of the influence of species, dose, and exposure route on perchloroethylene pharmacokinetics. J. Toxicol. Environ. Health. 44:301-317.

Dobrev, I., M.E. Andersen and R.S.H. Yang. 2001. Assessing interaction thresholds for trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane using gas uptake studies and PBPK modeling. Arch. Toxicol. 75:134-144.

Dobrev, I., M.E. Andersen and R.S.H. Yang. 2002. *In silico* toxicology: Simulating interaction thresholds for human exposure to mixtures of trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane. Environ. Health Perspect. 110(10):1031-1039.

el-Masri, H.A., R.S. Thomas, G.R. Sabados et al. 1996a. Physiologically based pharmacokinetic/pharmacodynamic modeling of the toxicologic interaction between carbon tetrachloride and Kepone. Arch Toxicol. 70(11):704-713.

el-Masri, H.A., A.A. Constan, H.S. Ramsdell and R.S.H. Yang. 1996b. Physiologically based pharmacodynamic modeling of an interaction threshold between trichloroethylene and 1,1-dichloroethylene in Fischer 344 rats. Toxicol. Appl. Pharmacol. 141:124-132.

Fenske, R.A., C. Lu, D. Barr and L. Needham. 2002. Children's exposure to chlorpyrifos and parathion in an agricultural community in central Washington State. Environ. Health Perspect. 110(5):549-553.

Fisher, J.W. 1993. Estimating the risks of liver and lung cancer in humans exposed to trichloroethylene using a physiological model. Toxicol. Lett. 68(1-2):127-129.

Fisher, J.W. and B.C. Allen. 1993. Evaluating the risk of liver cancer in humans exposed to trichloroethylene using physiological models. Risk Anal. 13(1):87-95.

Fisher, J.W., T.A. Whittaker, D.H. Taylor, H.J. Clewell III and M.E. Andersen. 1989. Physiologically based pharmacokinetic modeling of the pregnant rat: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol. Appl. Pharmacol. 99:395-414.

Fisher, J.W., T.A. Whittaker, D.H. Taylor, H.J. Clewell III and M.E. Andersen. 1990. Physiologically based pharmacokinetic modeling of the lactating rat and nursing pup: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol. Appl. Pharmacol. 102:497-513.

Fisher, J.W., M.L. Gargas, B.C. Allen and M.E. Andersen. 1991. Physiologically based pharmacokinetic modeling with trichloroethylene and its metabolite, trichloroacetic acid, in the rat and mouse. Toxicol. Appl. Pharmacol. 109:183-195.

Franks, N.P. and W.R. Lieb. 1994. Molecular and cellular mechanisms of general anesthesia. Nature. 367:607-614.

Gearhart, J.M., C. Seckel and A. Vinegar. 1993. *In vivo* metabolism of chloroform in B6C3F1 mice determined by the method of gas uptake: The effects of body temperature on tissue partition coefficients and metabolism. Toxicol. Appl Pharmacol. 119:258-266.

Gearheart, J.M., G.W. Jepson, H.J. Clewell, M.E. Andersen and R.B. Conolly. 1994. Physiologically based pharmacokinetic model for the inhibition of acetylcholinesterase by organophosphate esters. Environ. Health Perspect. 102(Suppl. 11):51-60.

Gentry, P.R., D.E. Hack, L. Haber, A. Maier and H.J. Clewell III. 2002. An approach for the quantitative consideration of genetic polymorphism data in chemical risk assessment: Examples with warfarin and parathion. Toxicol. Sci. 70:120-139.

Haddad, S., G. Charest-Tardif and K. Krishnan. 2000. Physiologically based modeling of the maximal effect of metabolic interactions on the kinetics of components of complex chemical mixtures. J. Toxicol. Environ. Health A. 61(3):209-223.

Kedderis, G.L. 1997. Extrapolation of *in vitro* enzyme induction data to humans *in vivo*. Chem. Biol. Interact. 107:109-121.

Kedderis, G.L. and S.D. Held. 1996. Prediction of furan pharmacokinetics from hepatocyte studies: Comparison of bioactivation and hepatic dosimetry in rats, mice and humans. Toxicol. Appl. Pharmacol. 140:124-130.

Kedderis, G.L. and J.C. Lipscomb. 2001. Application of *in vitro* biotransformation data and pharmacokinetic modeling to risk assessment. Toxicol. Ind. Health. 17:315-321.

Klein, M.T., G. Hou, R. Quann et al. 2002. BioMOL: A computer-assisted biological modeling tool for complex chemical mixtures and biological processes at the molecular level. Environ. Health Perspect. 110(Suppl. 6):1025-1029.

Koizumi, A. 1989. Potential of physiologically based pharmacokinetics to amalgamate kinetic data of trichloroethylene and tetrachloroethylene obtained in rats and man. Br. J. Ind. Med. 46:239-249.

Koizumi, A., M. Kumai and M. Ikeda. 1982. *In vivo* suppression of 1,1,1-trichloroethane metabolism by co-administered tetrachloroethylene: an inhalation study. Bull. Environ. Contam. Toxicol. 29:196-199.

Kousba, A.A., T.S. Poet and C. Timchalk. 2003. Characterization of the *in vitro* kinetic interaction of chlorpyrifos-oxon with rat salivary cholinesterase: A potential biomonitoring matrix. Toxicology. 188:219-232.

Levesque, B., P. Ayotte, R. Tardif et al. 2000. Evaluation of the health risk associated with exposure to chloroform in indoor swimming pools. J. Toxicol. Environ. Health A. 61:225-243.

Liao, K.H., I. Dobrev, J.E. Dennison Jr. et al. 2002. Application of biologically based computer modeling to simple or complex mixtures. Environ. Health Perspect. 110(Suppl. 6):957-963.

Lambert, J.C. and J.C. Lipscomb. 2007. Mode of action as a determining factor in human health risk assessment of chemical mixtures. Regul. Toxicol. Pharmacol. 49(3):183-194.

Lipscomb, J.C. and C.M. Garrett. 1998. Effect of organ procurement conditions on cytochrome P-450 activity in rat liver microsomes. In Vitro Mol. Toxicol. 11(3):265-270.

Lipscomb, J.C. and G.L. Kedderis. 2002. Incorporating human interindividual biotransformation variance in health risk assessment. Sci. Total Environ. 288:13-21.

Lipscomb, J.C., C.M. Garrett and J.E. Snawder. 1997. Cytochrome P450-dependent metabolism of trichloroethylene: Interindividual differences in humans. Toxicol. Appl. Pharmacol. 142:311-318.

Lipscomb, J.C., J.W. Fisher, P.D. Confer and J.Z. Byczkowski. 1998. *In vitro* to *in vivo* extrapolation for trichloroethylene metabolism in humans. Toxicol. Appl. Pharmacol. 152:376-387.

Lipscomb, J.C., L.K. Teuschler, J.C. Swartout, C.A.F. Striley and J.F. Snawder. 2003a. Variance of microsomal protein and cytochrome P450 2E1 and 3A forms in adult human liver. Toxicol. Mech. Methods. 13:45-51.

Lipscomb, J.C., L.K. Teuschler, J.C. Swartout, D. Popken, T. Cox and G.L. Kedderis. 2003b. The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans. Risk Anal. 23:1221-1238.

Loizou, G.D. 2001. The application of physiologically based pharmacokinetic modelling in the analysis of occupational exposure to perchloroethylene. Toxicol. Lett. 124:59-69.

McKone, T.E. 1993. Linking a PBPK model for chloroform with measured breath concentrations in showers: Implications for dermal exposure models. J. Exposure Anal. Environ. Epi. 3(3):339-365.

Mehendale, H.M. 2005. Tissue repair: An important determinant of final outcome of toxicant-induced injury. Toxicol. Pathol. 33:41-51.

Mumtaz, M.M. and P.R. Durkin. 1992. A weight of evidence approach for assessing interactions in chemical mixtures. Toxicol. Ind. Health. 8:377-406.

Mumtaz, M.M., K.A. Poirier and J.T. Colman. 1997. Risk assessment for chemical mixtures: fine tuning the hazard index approach. J. Clean Technol. Environ. Toxicol. Occup. Med. 6:189-204.

Mumtaz, M.M., C.T. DeRosa, J. Groten, V.J. Feron, H. Hansen and P.R. Durkin. 1998. Estimation of toxicity of chemical mixtures through modeling of chemical interactions. Environ Health Perspect. 106(Suppl. 6):1353-1360.

Poet, T.S., R.A. Corley, K.D. Thrall et al. 2000. Assessment of the percutaneous absorption of trichloroethylene in rats and humans using MS/MS real-time breath analysis and physiologically based pharmacokinetic modeling. Toxicol. Sci. 56:61-72.

Poet, T.S., K.K. Weitz, R.A. Gies et al. 2002. PBPK modeling of the percutaneous absorption of perchloroethylene from a soil matrix in rats and humans. Toxicol. Sci. 67:17-31.

Qiao, D., F.J. Seidler and T.A. Slotkin. 2001. Developmental neurotoxicity of chlorpyrifos modeled *in vitro*: Comparative effects of metabolites and other cholinesterase inhibitors on DNA synthesis in PC12 and C6 cells. Environ. Health Perspect. 109(9):909-913.

Rao, H.V. and D.R. Brown. 1993. A physiologically based pharmacokinetic assessment of tetrachloroethylene in ground water for a bathing and showering determination. Risk Anal. 13(1):37-49.

Reisfeld, B. and R.S.H. Yang. 2004. A reaction network model for CYP2E1-mediated metabolism of toxicant mixtures. Environ. Toxicol. Pharmacol. 18:173-179.

Reitz, R.H., J.N. McDougal, R.J. Nolan and A.M. Schumann. 1988. Physiologically based pharmacokinetic modeling with methylchloroform: Implications for interspecies, high dose/low dose, and dose route extrapolations. Toxicol. Appl. Pharmacol. 95:185-199.

Reitz, R.H., M.L. Gargas, A.L. Mendrala and A.M. Schumann. 1996. *In vivo* and *in vitro* studies of perchloroethylene metabolism for physiologically based pharmacokinetic modeling in rats, mice, and humans. Toxicol. Appl. Pharmacol. 136:289-306.

Roy, A., C.P. Weisel, P.J. Lioy and P.G. Georgopoulos. 1996. A distributed parameter physiologically-based pharmacokinetic model for dermal and inhalation exposure to volatile organic compounds. Risk Anal. 16(2):147-160.

Sato, A., T. Nakajima, Y. Fujiwara and N. Murayama. 1977. A pharmacokinetic model to study the excretion of trichloroethylene and its metabolites after an inhalation exposure. Br. J. Ind. Med. 34:55-63.

Sato, A., K. Endoh, T. Kaneko and G. Johanson. 1991. A simulation study of physiological factors affecting pharmacokinetic behavior of organic solvent vapours. Br. J. Ind. Med. 48(5):342-347.

Shibata, A., K. Morita, T. Yamashita, H. Kayama and I. Ueda. 1991. Anesthetic-protein interaction: Effects of volatile anesthetics on the secondary structure of poly-(l-lysine). J. Pharm. Sci. 80(11):1037-1041.

Snawder, J.E. and J.C. Lipscomb. 2000. Interindividual variance of cytochrome P450 forms in human hepatic microsomes: Correlation of individual forms with xenobiotic metabolism and implications in risk assessment. Regul. Toxicol. Pharmacol. 32:200-209.

Sultatos, L.G. 1990. A physiologically based pharmacokinetic model of parathion based on chemical-specific parameters determined *in vitro*. J. Amer. Coll. Toxicol. 9(6):611-619.

Tardif, R. and G. Charest-Tardif. 1999. The importance of measured end-points in demonstrating the occurrence of interactions: A case study with methylchloroform and m-xylene. Toxicol Sci. 49:312-317.

Thrall, K.D. and T.S. Poet. 2000. Determination of biokinetic interactions in chemical mixtures using real-time breath analysis and physiologically based pharmacokinetic modeling. J. Toxicol. Environ. Health A. 59(8):653-670.

Timchalk, C., A. Kousba and T.S. Poet. 2002a. Monte Carlo analysis of the human chlorpyrifos-oxonase (PON1) polymorphism using a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model. Toxicol Lett. 135:51-59.

Timchalk, C., R.J. Nolan, A.L. Mendrala, D.A. Dittenber, K.A. Brzak and J.L. Mattsson. 2002b. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicol Sci. 66:34-53.

Ueda, I. and H. Kamaya. 1984. Molecular mechanisms of anesthesia. Anesth. Analg. 63:929-945.

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

U.S. EPA. 1989. Risk Assessment Guidance for Superfund, Volume I (RAGS), Human Health Evaluation Manual, Part A, Interim Final. December. OSWER 9285.7-02B, EPA/540/1-89/002. Office of Emergency and Remedial Response, U.S. Environmental Protection Agency, Washington, DC. Available at http://www.epa.gov/superfund/programs/risk/ragsa/index.htm.

U.S. EPA. 1998. Rule Writer's Guide to Executive Order 13045: Guidance for Considering Risks to Children During the Establishment of Public Health-Based and Risk-Based Standards. U.S. Environmental Protection Agency, Office of Children's Health Protection. Washington, DC. November.

U.S. EPA. 2000a. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. U. S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/R-00/002. Available at http://www.epa.gov/ncea/raf/pdfs/chem_mix/chem_mix_08_2001.pdf.

U.S. EPA. 2000b. Cumulative Risk: A Case Study of the Estimation of Risk from 24 Organophosphate Pesticides. U.S. Environmental Protection Agency, Office of Pesticide Programs, Health Effects Division, Washington, DC. November 9. Available at http://www.epa.gov/scipoly/sap/meetings/2000/december/sap_casestudy2.pdf.

U.S. EPA. 2002a. Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC. January 14. Available at http://www.epa.gov/oppfead1/trac/science/#common.

U.S. EPA. 2002b. Organophosphate Pesticides: Revised OP Cumulative Risk Assessment. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC. Available at http://www.epa.gov/pesticides/cumulative/rra-op/.

U.S. EPA. 2003a. Physiologically-Based Pharmacokinetic/Pharmacodynamic Modeling: Preliminary Evaluation and Case Study for the n-Methyl Carbamate Pesticides. U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, DC. November 10. Available at http://www.epa.gov/scipoly/sap/meetings/2003/december11/pbpksapfinal.pdf.

U.S. EPA. 2003b. A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: Physiologically-based Pharmacokinetic/ Pharmacodynamic Modeling: Preliminary valuation and Case Study for the N-Methyl Carbamate Pesticides: A consultation. Presented at FIFRA Scientific Advisory Panel Meeting, Sheraton Crystal City Hotel, Arlington, VA. December 11-12. SAP Minutes No. 2003-06. Available at http://www.epa.gov/scipoly/sap/meetings/2003/december11/meetingminutes.pdf.

U.S. EPA. 2003c. Framework for Cumulative Risk Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/P-02/001F. Available at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=54944.

U.S. EPA. 2006. Exposures and Internal Doses of Trihalomethanes in Humans: Multi-Route Contributions from Drinking Water. Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. EPA/R-06/087.

U.S. EPA. 2007a. Revised *N*-Methyl Carbamate Cumulative Risk Assessment U.S. Environmental Protection Agency Office of Pesticide Programs. Docket # EPA-HQ-OPP-2007-0935 http://www.regulations.gov/fdmspublic/component/main?main=DocketDetail&d=EPA-HQ-OPP-2007-0935

U.S. EPA. 2007b. Integrated Risk Information System (IRIS). Online. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available at www.epa.gov/iris.

Vogel, J.S., G.A. Keating II and B.A. Buchholz. 2002. Protein binding of isofluorophate *in vivo* after coexposure of multiple chemicals. Environ. Health Perspect. 110(Suppl. 6):1031-1036.

Ward, R.C., C.C. Travis, D.M. Hetrick, M.E. Andersen and M.L. Gargas. 1988. Pharmacokinetics of tetrachloroethylene. Toxicol. Appl. Pharmacol. 93:108-117.

Whyatt, R.M., D.E. Camann, P.L. Kinney et al. 2002. Residential pesticide use during pregnancy among a cohort of urban minority women. Environ. Health Perspect. 110(5):507-514.

Wilson, J.D. and J. Knaak. 1994. Health risk assessment of tetrachloroethylene using a physiologically-based pharmacokinetic model. Abstr. Pap. Am. Chem. Soc. 207:62-ENVR.

Yang, R.S.H., K.H. Liao and B. Reisfeld. 2004a. The integration of computer modeling and experimental toxicology for the study of chemical mixtures and multiple stressors. Arch. Complex Environ. Factors. In press.

Yang, R.S.H., H.A. El-Masri, R.S. Thomas et al. 2004b. Chemical mixture toxicology: From descriptive to mechanistic, and going on to *in silico* toxicology. Environ. Toxicol. Pharmacol. 18:65-81.