APPENDIX D
EXTERNAL PANEL REVIEW:
Method of Analysis to Perform a Tissue-Based Cumulative Risk Assessment for Mixtures of Chemicals (NCEA-C-1602)

The following panelists were identified and retained by Versar, Inc.

Richard J. Bull, Ph.D.
MoBull Consulting
Richland, WA

Harvey Clewell (Chair)
CIIT Centers for Health Research
Research Triangle Park, NC

Gary L. Ginsberg, Ph.D.
Connecticut Department of Public Health
Hartford, CT

Margaret MacDonell, Ph.D.
Argonne National Laboratory
Argonne, IL

Moiz M. Mumtaz, Ph.D.
Agency for Toxic Substances and Disease Registry (ATSDR)
Chamblee, GA

Clifford P. Weisel, Ph.D.
Environmental & Occupational Health Sciences Institute (EOHSI)/UMDNJ
Piscataway, NJ

I. PRE-MEETING COMMENTS

Following dissemination of the draft document and before the convening of the panel review, comments were solicited from panelists. Two sets of comments were received (Clewell and Ginsberg); those pre-meeting comments were discussed by the review panel. Where concerns remained, those concerns were treated in the final review comments. As such, there are no EPA responses to pre-meeting comments.

1.A. Harvey Clewell

(1) Method of Analysis to Perform a Tissue-Based Cumulative Risk Assessment for Mixtures of Chemicals (NCEA-C-1602)

1. Does the document clearly distinguish interactions at the level of cumulative risk assessment and pharmacokinetics? Does the document present the benefits of employing tissue dose, rather than environmental concentration as the basis for cumulative risk assessment?

Reviewer Response: The document does provide a reasonably clear distinction between interactions as defined in default cumulative risk assessment and interactions in pharmacokinetics, although there are some awkward sentences, e.g.:

"Form [stet] 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. "

I think this sentence should be expanded into a paragraph that takes the necessary time to lay out the idea it is being attempted to convey.

The document does a good job of describing the benefits of using tissue dose, but it would be strengthened if examples were presented where considering tissue dose made a difference.

2. The decision point for continuing to develop a comprehensive cumulative risk assessment (a PBPK-based approach and assessment) is presented in Step 4 of the analysis plan. It is based on the assumption of chemical additivity, and includes a Hazard Index type analysis coupled with an uncertainty factor intended to address interactions (in the broad definitional sense). This point has drawn appreciable comment previously. The intent of the document is to communicate considerations undertaken in the series of choices relating to resource expenditure for CRA. Please comment on the clarity and strength of the rationale presented and propose other considerations you feel may be useful to incorporate. Please comment specifically on the proposed 1000-fold uncertainty factor and on conditions when it may be warranted. Below is an example comment and response relating to this point:

Proposed "Rules of Thumb" for Estimating Threshold of Toxicological Interactions

<u>INTERNAL REVIEWER COMMENT</u>: *"Thus, for practical purpose of conducting CRA" Interesting rule, but the stated rationale (stated at the end on page 19) is pretty weak. The screening calculation might make more sense if stated in terms of an average hazard index (i.e., sum of each chemical's exposure potential /RfD, and that sum then divided by N) or relative potency or some other normalizing method, then applying an additional screening factor (like an uncertainty factor or margin of safety factor) to account for potential synergistic interactions. One could also evaluate the potential for antagonism and a lower level of hazard for a mixture. A fixed screening factor for synergistic interactions (100 or 1000) could be consistently applied for all CAGs, but I think a CAG specific factor would be better. The case by case factor could include some weighting for the number in the group, although I think better based upon what we know about the chemicals' metabolism or other binding, what we know about*

competitive inhibition/binding at different dose levels for the CAG chemicals, or about chemical reactivity for the chemicals in the CAG. A similar method could apply for a cancer index using potential exposure over the 10^6 risk level (from slope factors or linear extrapolations from the NOAELs as the POD 10%).
RESPONSE: This is a very good comment; likewise it was echoed in those from the other reviewer. This issue (how best to develop a method to cull out potential scenarios for which a Comprehensive Cumulative Risk Assessment should be performed) will be raised for discussion at the level of external review. It may be that such a point (when to not do such an assessment) should not be specifically "codified." but be left to be addressed on a case-by-case basis. This issue will be forwarded to the external review panel for comment.

Reviewer Response: I agree with the reviewer above. The rule of thumb should be characterized in terms of the hazard index for the mixture, and the numerical value of the "screening factor" for a given mixture should be left to scientific judgement.

3. Are you aware of any additional information which can be used to improve the present draft report?

Reviewer Response: No.

4. Are there assumptions or uncertainties being made in this exercise that are not articulated?

Reviewer Response: No.

5. Are the figures and tables informative? Would revision of the tables or figures improve the clarity of the report or its conclusions? If so, what are they?

Reviewer Response: The figures and tables are adequate.

6. Are there important publications missing from the reference section?

Reviewer Response: I think the report would be improved by doing a literature search and adding examples of interaction modeling that demonstrate its value.

7. What additional information would you like to see presented?

Reviewer Response: Examples of modeling of interactions and how the modeling provided a quantitative understanding of the impact of the interaction on cumulative risk.

8. Please comment on the overall quality of the report and analysis. What is your overall evaluation of the scientific content, readability and utility of the draft report? Do

you have any suggestions relative to structure or content that would improve the quality of this draft report?

> **Reviewer Response:** For the most part, the report is well written and scientifically sound. It should be useful as a an initial source of discussion on the merits of tissue dosimetry in cumulative risk assessment. It sometimes feels rather shallow, however, because there are no concrete examples of the successful use of tissue dosimetry to improve a cumulative risk assessment. I believe such examples exist for chemicals other than those discussed in the report, although they admittedly are for high exposure interactions (e.g., TCE/VDC, BTEX).
>
> There is one scientific inaccuracy I noticed: the reaction of OPs with AChE is not, in general, irreversible, as suggested on p. 43. In fact, characterization of the relative rates of regeneration (reversible binding) and aging (irreversible binding) is an important aspect of the identification of the more dangerous OPs (e.g., soman).

1.B. Gary L. Ginsberg, Ph.D.

General Comments

This document presents some interesting arguments on how to screen multiple chemical exposures and determine whether an extensive PBPK modeling assessment would be needed to evaluate cumulative risk. In this regard it is useful. However, it is written more like a white paper than a framework or general guidance document and so would likely to be difficult to follow for the average risk assessor. The ultimate user of this document is unclear (researchers/modelers? headquarters risk assessors involved in policy? Risk assessors in the field?). It would be helpful at the outset to describe the intended audience and how they might consider using the approach. Regardless of the audience, a summary of the proposed approach which highlights the key analytical phases and steps is needed. The document is wordy and somewhat redundant with the purpose of individual sections relative to the overall flow not always clear. Starting with a flow diagram or outline of the proposed approach (e.g., the 10 steps divided into 2 phases with brief outline of purpose of each step) would help keep the various sections in perspective. Then at the end, a section is needed which summarizes the overall approach to tie it together for the intended user. For example, it might state that the screening level approach can be conducted by most risk assessors to quickly evaluate whether interactions are possible and whether a more quantitative (modeling) approach is needed. This then would require recruitment of analysts which can gather the PK information, build interaction models, etc. etc. Right now, the document makes a number of good suggestions but does not do a good job of presenting them as an integrated and practical approach.

Charge Questions and Reviewer Responses

1. Does the document clearly distinguish interactions at the level of cumulative risk assessment and pharmacokinetics? Does the document present the benefits of employing tissue dose, rather than environmental concentration as the basis for cumulative risk assessment?

> **Reviewer Response:** The document spends considerable effort distinguishing between interactions than can be judged based upon additivity of effect vs. those that occur on a biochemical or toxicokinetic basis. While this generally comes across, it can be more clearly described in Section 1. For example, the 2nd paragraph of page 7 thru page 8 is the place where this distinction is made for the first time. However, the paragraph starts out with other concepts (interactions that are passive/partitioning vs. active – metabolic) that are an aside to the key point that the interaction can be on a PD basis (additivity of effect such as with the OPs) or on a PK basis (interference with metabolic activation, detoxification, clearance, binding, etc.). From such an introduction, one could then go on to state how these types of interactions are different and need to be analyzed differently (e.g., one with RfD approach, one with PBPK analysis) but that both types of interactions need to be considered jointly as part of the overall CRA. Setting up the differences while also explaining the need for a common analytical framework is critical to the success of the rest of the document. Right now the reader has to do too much work to figure out the implications of the distinction that is being made on pages 7-8. I don't think this is clarified to a satisfactory extent at later points where the difference is brought up again. However, I must add that the 2 types of mixtures examples as discussed on pages 13-14, the OPs vs. the chlorinated solvents, does help clarify the distinction in that they exemplify the two types of interactions quite well. Some framing verbiage would help solidify that understanding (e.g., "The two case study mixtures were selected to exemplify ……, OPs on the one hand represent ……, while the chlorinated water contaminants represent the other type of interaction at the PK level. This document uses these different examples to show that different types of interactions can be analyzed jointly within a unified framework").
>
> The second part of this charge question, whether the case is adequately made for the benefits of analyzing at the tissue dose rather than environmental concentration level. The case for this is argued fairly strongly with at times overzealous language (e.g., - page 4 - "wonderful", "the best"). However, it is still somewhat unclear whether the tissue (PBPK) level of analysis is needed for CRAs involving interaction at the PD level. If this is the case (and it may well be), it is not argued well in this document. In contrast, the need for internal dose resolution for PK interactions is clear.

2. The decision point for continuing to develop a comprehensive cumulative risk assessment (a PBPK-based approach and assessment) is presented in Step 4 of the

analysis plan. It is based on the assumption of chemical additivity, and includes a Hazard Index type analysis coupled with an uncertainty factor intended to address interactions (in the broad definitional sense). This point has drawn appreciable comment previously. The intent of the document is to communicate considerations undertaken in the series of choices relating to resource expenditure for CRA. Please comment on the clarity and strength of the rationale presented and propose other considerations you feel may be useful to incorporate. Please comment specifically on the proposed 1000-fold uncertainty factor and on conditions when it may be warranted. Below is an example comment and response relating to this point:

Proposed "Rules of Thumb" for Estimating Threshold of Toxicological Interactions

INTERNAL REVIEWER COMMENT "Thus, for practical purpose of conducting CRA" Interesting rule, but the stated rationale (stated at the end on page 19) is pretty weak. The screening calculation might make more sense if stated in terms of an average hazard index (i.e., sum of each chemical's exposure potential /RfD, and that sum then divided by N) or relative potency or some other normalizing method, then applying an additional screening factor (like an uncertainty factor or margin of safety factor) to account for potential synergistic interactions. One could also evaluate the potential for antagonism and a lower level of hazard for a mixture. A fixed screening factor for synergistic interactions (100 or 1000) could be consistently applied for all CAGs, but I think a CAG specific factor would be better. The case by case factor could include some weighting for the number in the group, although I think better based upon what we know about the chemicals' metabolism or other binding, what we know about competitive inhibition/binding at different dose levels for the CAG chemicals, or about chemical reactivity for the chemicals in the CAG. A similar method could apply for a cancer index using potential exposure over the 10^6 risk level (from slope factors or linear extrapolations from the NOAELs as the POD 10%).

RESPONSE: This is a very good comment; likewise it was echoed in those from the other reviewer . This issue (how best to develop a method to cull out potential scenarios for which a Comprehensive Cumulative Risk Assessment should be performed) will be raised for discussion at the level of external review. It may be that such a point (when to not do such an assessment) should not be specifically "codified", but be left to be addressed on a case-by-case basis. This issue will be forwarded to the external review panel for comment.

> **Reviewer Response:** This screening approach is both intriguing and troubling. The attraction to it is that it offers a unified way to screen different types of interaction mechanisms for potential likelihood of occurrence. The problem is that one approach may not fit different interaction mechanisms. The RfD/N additivity approach comes out of the common PD MOA – additivity mechanism in which each ingredient in the mixture has a common PD target/endpoint (e.g., brain AChE inhibition). In this case, a simplistic hazard equation (exposure dose/modified RfD) makes some sense in that the RfD for single compounds is set to be below an effects level for even sensitive individuals and lowering the RfD to take into account interacting chemicals that have the same MOA has

some rationale. It may be a conservative default if the various chemicals have similar potency. One gets nervous about this approach for similarly targeted chemicals when one considers the malathion/dicrotophos comparison presented on pages 28-29. Here are similarly acting OPs which have vastly different dose response for the same endpoint. If we are evaluating an interaction between these 2 OPs, does it make sense to only lower the malathion RfD (the less potent ingredient) by a factor of 2? One might guess that the high potency of dicrotophos might cause it to be more influential in an interaction scenario and so should be more heavily weighted. Perhaps whats needed is an approach which weights each ingredient's contribution to the interaction based upon its potency and its dose level in the system. If dicrotophos is present at levels far below its RfD, then it likely doesn't matter that its so potent. Thus, rather than adding malathion and dicrotophos together as two equally important constituents (1+1=2) and then dividing each RfD by 2, perhaps one should consider the dicrotophos contribution relative to the malathion contribution as follows:

(Malathion RfD/Dicrotophos RfD) * (Dicrotophos dose level/ Dicrotophos RfD)

If Malathion RfD is 1000x greater than the Dicrotophos RfD and the dicrotophos dose is 1000x lower than its RfD, then the above equation yields 1 and the dichrotophos contribution to the cumulative interaction would equal that contributed by malathion and you could divide the malathion RfD by 2 (N=2) as in the proposed screening method. However, if the dicrotophos exposure level is 0.1 of the RfD, then the malathion RfD would be divided by 1 (for malathion) + 100 (from above equation for dicrotophos contribution) (N = 101). Obviously this can become more complicated and unwieldy for ternary or higher order interactions and so one needs a simplifying assumption that the potency adjustment is only needed for the more potent ingredients and only if they exceed a given ingredient's potency (lower RfD) by 3x or more. Thus, if you have 5 OPs in the mixture with 3 having similar potency and 2 being considerably more potent, you would only need to run the above equation twice to calculate how much RfD lowering is contributed by these 2 more potent ingredients.

The above discusses issues with the screening approach for chemicals acting via similar PD MOAs. However, this approach may not be so relevant for chemicals which interact strictly via PK mechanisms (e.g., the chlorinated solvents interacting at the level of CYP2E1). In this case, there may be no relationship between a chemical's RfD and its ability to compete for throughput via 2E1. For example, a chemical may have a high RfD but also a high affinity for 2E1 which may make it a stronger metabolic interactor than a chemical with a low RfD but weak affinity for 2E1. Thus, for PK-interacting chemicals, screening on the basis of RfD/N may have little relevance to what is going on biochemically, especially since these chemicals may have different PD targets and MOAs (e.g., one solvent affecting CNS while other affects liver). The example of fenitrothion and parathion interaction is a good example of the fact that a chemical does not have to exert a measureable toxic effect (only a biochemical effect) to synergize

another chemical. Fenitrothion ties up peripheral carboxylesterases so that at doses well below those required for fenitrothion AChE inhibition, a synergistic interaction with parathion is set up.

It may well be that in most cases, the RfD is set low enough so that not only are risks for toxicity averted but also there is no biochemical perturbation relevant to interaction with other chemicals at the RfD/N dose. However, the case for this needs to be explored and made stronger. For example, dioxin is a potent inducer of the CYP1A family which may cause interactions with other CYP1A family substrates. Do we know that RfD/N for dioxin (or PCBs) will make this type of biochemical/interaction mechanism moot?

I believe that evidence supporting this screening approach may be obtainable via interactive case studies which involve PBPK analyses exploring what goes on at high dose vs. what occurs at RfD-type doses. My guess would be that for simple competition interactions and protein binding interactions, the RfD/N approach would be a reasonably conservative screen. However, this may be less clear for enzyme induction interactive mechanisms, or perhaps for non-competitive inhibition mechanisms. The fenitrothion/parathion interaction mentioned above would be interesting to test in this regard.

This charge question also raises the issue of whether a default approach (NOAEL / 1000 fold UF) is a useful default in the case where an RfD is unavailable for a particular constituent. The idea is that a 1000x cumulative UF will create an RfD that is low enough to protect against the possibility of synergistic interactions. This approach does not appear to be well thought out. The RfD should be set based upon the relevance and strength of the underlying database. The greater the uncertainty, the larger the cumulative UF. RfDs are set based upon the dose-response assessment for individual chemicals and are not based upon whether they have an interaction with other chemicals. It would be preferable to stick with the standard risk assessment approach to RfD setting when starting from a NOAEL and not use a default factor that incorporates concerns over synergistic interaction. The analysis of interaction, whether screening or detailed, should be a separate step from dose-response assessment. Since IRIS RfDs do not incorporate a group interaction UF, to use one in the case of a missing RfD would create an apples and oranges situation in which the different types of RfDs would not be comparable.

It is important to note that the focus should not be on how much potentiation is possible in a synergistic interaction but whether the concentrations of the reactants are high enough for a meaningful interaction to take place. If this is so, then one moves beyond the screening level to Phase II in the 10 step process. Hopefully during this phase one can determine whether synergy is possible and to what extent.

If there is a particular concern over the possibility of synergy during the screening phase of the analysis (e.g., OPs that can interact the way that fenitrothion interacts with malathion) one could incorporate it into the RfD/N approach by increasing the value of N for synergistic chemicals in a manner similar to the dichrotophos example above. Such a method could be tested with known synergistic interactions on a pharmacokinetic level of analysis to document that the screening level approach is protective against synergists. Such analyses may show what additional factor (if any) is needed to modify N in the RfD/N paradigm to ensure protection against synergistic interactions.

In summary, I would recommend against adjustment of the way in which one goes from a NOAEL to an RfD but would rather adjust for synergistic interactions on a case specific basis by adjusting the value of N in RfD/N.

3. Are you aware of any additional information which can be used to improve the present draft report?

Reviewer Response: As stated above, the fenitrothion/parathion synergistic interaction is important to mention. The document should also describe other types of interactions (non-competitive inhibition, enzyme induction, etc) and how they can be brought within the CRA analytical framework.

4. Are there assumptions or uncertainties being made in this exercise that are not articulated?

Reviewer Response: No, nothing not already talked about.

5. Are the figures and tables informative? Would revision of the tables or figures improve the clarity of the report or its conclusions? If so, what are they?

Reviewer Response: As described above, a framework overview figure would be very helpful at the outset and a summary version at the end showing key decision points would also be helpful.

Figure 1 does not capture the interaction between OPs at the level of peripheral carboxylestases (CE). A prime example of this is the fenitrothion synergism of parathion. This type of interaction should be included in the chart.

Figure 2 might be improved by indicating the direction of the interaction at each step: e.g., if 1 inhibits 3 at level of 2E1 then there might be more of 3 available for GSH conjugation and renal toxicity. Without this context the figure can seem overwhelming and may not relate as clear a message regarding the importance of the interactions.. vis-à-vis the text.

6. What additional information would you like to see presented?

Reviewer Response: The paper lacks citations of PBPK modeling studies in which interactions were evaluated. I believe several studies have been published along these lines. Additional background information on the various types of PD and PK interactions possible would be helpful (e.g., interaction at level of competitive or non-competitive enzyme inhibition; at level of GSH depletion predisposing to second toxicant that needs GSH; at level of enzyme induction, etc.

7. Please comment on the overall quality of the report and analysis. What is your overall evaluation of the scientific content, readability, and utility of the draft report? Do you have any suggestions relative to structure or content that would improve the quality of this draft report?

Reviewer Response: For overall quality, readability and utility, see general comments above. To reiterate, the intended end user(s) should be stated up front, how and when they might use the document should also be stated, and then this should be kept in mind throughout the writing. This may enable it to become less of a white paper and more of an analytical framework document.

Some specific comments are as follows:

Section 1.1 –the lengthy quotes from the Hansen and Gilman memos do not appear to be necessary. The main points can be highlighted much more briefly, using selective quotes.

Page 16, large para – This paragraph addresses the problem of aggregating exposures across chemicals, each of which has its own data distribution. The discussion here seems too complex and misses what I think is the main option – a screening level approach where an upper bound value is used for each contaminant. If there are not signficiant interactions with these across the board upper bound concentrations, then there is little need to worry about the full data distributions for each analyte. There are a number of options for more refined analyses. One in particular that may be attractive is to run the interaction scenario 4 times (if there are 4 chemicals interacting in the mixture). Each run uses the 95^{th} percentile for one chemical and the average concentration for the other 3 chemicals when the first chemical is at its 95^{th} percentile. The point sampling approach on pages 16 to 17 is worth mentioning but appears to be impractical.

Page 19, bottom para – The issue of age-related variability is briefly discussed. Some contextual discussion is needed of the types of immaturities that may predispose to PK or PD interactions in early life as opposed to adults. One can cite the early life vs. adult OP studies showing greater sensitivity in young rats with mechanistic work demonstrating both PK and PD bases for the sensitivity

differences. This has obvious bearing on the potential for OP to OP interactions in young vs. old rats.

Page 27 – middle of page – "some number of components would be at a significant fraction of the NOAEL. These components would survive the initial analysis."

What is meant by a significant fraction? Several lines later it states that others may be present at levels below or near RfD/N and so could be excluded. This language is too vague. The screening level RfD/N approach needs to be clarified with regards to what exposure level (relative to RfD/N) would constitute a significant interaction potential.

Pages 36-38 and beyond – discussion of PBPK modeling of chemical interactions – this section mentions some key parameters that will be needed but does not describe how these parameters can come together to predict the outcome of the interaction – e.g., that the ratio of tissue concentration to Km is a key factor for competitive interactions involving M-M kinetics. It would be useful to have a table of the key interactive equations (and parameter definitions) for the major types of interactions: M-M enzyme competive, non-competitive; competition for protein binding sites (e.g., albumin, CEs), enzyme induction, etc.

Page 38, bottom: should describe some of the issues with recombinant CYP systems (e.g., expression systems may not also express electron transport enzymes or they may be in wrong proportions).

II. FINAL REVIEW COMMENTS

II.A. Document Overview

Dr. Lipscomb provided an overview of the document and reviewed the charge questions. He explained that pharmacokinetics is important in evaluating human exposure and refining the doses used in the dose-response relationship. It is important in cumulative risk and mixtures assessment because the internal doses of some components of the mixture can alter the tissue dosimetry of other components. Dr. Lipscomb provided as background information the fact that Congress mandated that EPA conduct a cumulative risk assessment for organophosphorous (OP) pesticides that share a common mode of action. Because of that mandate, the EPA's guidance determines that when chemicals have the same mode of action, one can use the dose-addition or response-addition approach to evaluate cumulative risk. One of EPA's challenges is to develop methods to estimate risks from environmental mixtures containing a variety of chemicals, often acting through different modes of action. PBPK modeling is a valuable tool for developing the approaches to conduct cumulative risk assessments, with internal tissue doses being a critical step in the process. Dr. Lipscomb suggested that the group should keep this important issue in mind when reviewing the first document.

Method of Analysis to Perform a Tissue-Based Cumulative Risk Assessment for Mixtures of Chemicals is a document that attempts to take a 10-step process, developed by the Office of Pesticide Programs under a Congressional mandate, and inform a cumulative risk assessor of the benefits and considerations that need to be recognized when the assessor applies the 10-step process. The document presents two examples of chemical mixtures, OP pesticides and volatile organic chemicals. Dr. Lipscomb stated that the key step is the decision point which is after Step 5 in the 10-step process. The document discusses comprehensive cumulative risk assessment which means a physiologically- or tissue-based approach. Dr. Lipscomb believes that it will be difficult to get to that stage of risk assessment due to the lack of reliable data. He stated that the analysis still needs to be done but there might be uncertainty in the outcome.

Dr. Lipscomb indicated that confusion may arise about the term "interaction," which in cumulative risk assessment means that the observed toxic response cannot be predicted by the addition of the doses of the individual components or responses from the addition of individual components. Pharmacokinetics can assist in considering metabolic interactions and estimating chemical concentrations in the target tissue.

Dr. Lipscomb explained that the document is intended for NCEA internal use. He reviewed the charge questions and highlighted that he would like comments regarding the decision point step of the process.

II.B. General Comments

Mr. Clewell requested general comments from the reviewers on the document, *Method of Analysis to Perform a Tissue-Based Cumulative Risk Assessment for Mixtures of Chemicals*. Dr. Richard Bull stated that the reaction network modeling references interrupt the flow of the document and could be made into footnotes. Dr. Gary Ginsberg recommended toning down the "overzealous" language (terms like "wonderful"). Dr. Moiz Mumtaz felt that the document did not reflect the EPA 2000 "Supplementary Guidance for Mixtures" document. Also, he felt that the approach for assessing 3-4 chemicals is not adequate for more realistic environmental mixtures containing hundreds of chemicals. Dr. Clifford Weisel felt that the section on pharmacodynamics was rushed and Dr. Bull agreed; they decided to address this issue in the responses to charge questions. Mr. Clewell agreed with the previous comments and added that there should be discussion of all the different levels of interactions that can occur, not just metabolic enzyme interactions. He also added that these metabolic interactions typically do not occur at low doses, which is needed for the assessment to be interesting in terms of evaluating interactive effects. Dr. Ginsberg agreed that it was important to point out that other pharmacokinetic interactions exist, but to state that the document focuses on the 10-step process. Dr. Mumtaz agreed that other types of interactions should be mentioned and acknowledged, but that it should be pointed out that they wouldn't be completely explained or solved in the document. Dr. Ginsberg did

not feel that the document was user-friendly and would like a flowchart of the 10-step process included up front in the document to help the reader understand where they are in the process as they move through the steps. Mr. Clewell agreed and would also like a diagram of the levels of interaction included.

II.C. Response to Charge Questions

1. Does the document clearly distinguish interactions at the level of cumulative risk assessment and pharmacokinetics?

> **Reviewer Response:** Yes, it does, for the most part, but more discussion in the early part of the document would be helpful (section 1.6). Examples of published interactions and their impact would also help. E.g., mention of interaction threshold dose studies. Case studies could be used to demonstrate the different levels of interactions.
>
> There needs to be some discussion of other levels of interactions (e.g., pharmacodynamic) between the two extremes currently identified in the document.
>
> **EPA Response:** Section 1.6 has been substantially expanded. Examples of some published interactions have been added. The second paragraph contains a description of a work by Dobrev and colleagues that demonstrates interactions thresholds. This constitutes one case study where an interaction threshold has been determined.
>
> Some discussion of other levels (types) of interactions has also been added to section 1.6. The fifth paragraph contains the example of piperonyl butoxide (PBO) and a Detoxicated insecticide. Here, PBO exerts an effect which is unlikely associated with the toxicity of PBO, but which markedly decreases the ability of the organism to metabolize the active insecticide. Also, the example of thioacetamide and carbon tetrachloride has been included in paragraph 6. This example demonstrates how pretreatment with one toxic compound can be protective against subsequent and otherwise lethal doses of another compound. Finally, paragraph 8 contains an example indicated in the reviewers' comments to charge question 3. The OP inhibition data from the Chambers et al. and Cohen et al. publications have been included in this paragraph.

Does the document present the benefits of employing tissue dose, rather than environmental concentration as the basis for cumulative risk assessment?

> **Reviewer Response:** A better case is made for the value of tissue dose for pharmacokinetic interactions than for its value for cumulative risk assessment. Need to include a hypothetical case study of receptor interaction in section 2.3.1.4.

EPA Response: The modifications made in response to Charge question 1 largely address this comment. The additions specifically include measures of internal dose (especially the example by Dobrev and colleagues) for use in interactions as well as in cumulative risk assessment. The report has been challenged to include characterizations of receptor interactions in multiple locations have been suggested by one particular reviewer. We feel that the document adequately conveys its general points without the inclusion of a receptor interaction example.

2. The decision point for continuing to develop a comprehensive cumulative risk assessment (a PBPK-based approach and assessment) is presented in Step 4 of the analysis plan. It is based on the assumption of chemical additivity, and includes a Hazard Index type analysis coupled with an uncertainty factor intended to address interactions (in the broad definitional sense). This point has drawn appreciable comment previously. The intent of the document is to communicate considerations undertaken in the series of choices relating to resource expenditure for CRA. Please comment on the clarity and strength of the rationale presented and propose other considerations you feel may be useful to incorporate. Please comment specifically on the proposed 1000-fold uncertainty factor and on conditions when it may be warranted. Below is an example comment and response from the Internal Review relating to this point:

Internal Review Comment, Proposed "Rules of Thumb" for Estimating Threshold of Toxicological Interactions

INTERNAL REVIEWER COMMENT "Thus, for practical purpose of conducting CRA" Interesting rule, but the stated rationale (stated at the end on page 19) is pretty weak. The screening calculation might make more sense if stated in terms of an average hazard index (i.e., sum of each chemical's exposure potential /RfD, and that sum then divided by N) or relative potency or some other normalizing method, then applying an additional screening factor (like an uncertainty factor or margin of safety factor) to account for potential synergistic interactions. One could also evaluate the potential for antagonism and a lower level of hazard for a mixture. A fixed screening factor for synergistic interactions (100 or 1000) could be consistently applied for all CAGs, but I think a CAG specific factor would be better. The case by case factor could include some weighting for the number in the group, although I think better based upon what we know about the chemicals' metabolism or other binding, what we know about competitive inhibition/binding at different dose levels for the CAG chemicals, or about chemical reactivity for the chemicals in the CAG. A similar method could apply for a cancer index using potential exposure over the 10^6 risk level (from slope factors or linear extrapolations from the NOAELs as the POD 10%).

RESPONSE: This is a very good comment; likewise it was echoed in those from the other reviewer . This issue (how best to develop a method to cull out potential scenarios for which a Comprehensive Cumulative Risk Assessment should be performed) will be raised for discussion at the level of external review. It may be that such a point (when to not do such an assessment) should not be specifically "codified",

but be left to be addressed on a case-by-case basis. This issue will be forwarded to the external review panel for comment.

Reviewer Response:

A. Conceptually, such a screening rule of thumb is a good idea. Practically, the difficulty is knowing the relationship of the RfD and the interaction threshold. Should use target organ toxicity data, not most sensitive NOAEL, since the latter may not be for same site.
B. A default factor of 1000 should not be applied; instead an RfD equivalent should be derived for the appropriate effect, as explained in the TTD document. Secondary endpoints (e.g., developmental) should also be considered.
C. The document should make the point that the interaction threshold may be determined by pharmacokinetic factors (for example, saturation of enzyme systems and enzyme inhibition or induction), and not a toxicity threshold. The interaction threshold may determined by data on the dose/response for the induction of an enzyme, for example, but this should not be confused with a threshold for a toxic effect.
D. Case by case informed scientific judgment based on knowledge of the mechanism of action is necessary. The proposed rule of thumb may be valuable for the organophosphate common mechanism group, but it is questionable to apply it for metabolic interactions, without data on the relationship of the dose-responses for metabolic interactions and toxicity.

EPA Response:

A. The proposed rule of thumb has been removed and the issue has been characterized as one that should be undertaken on a case-by-case basis.
B. The default factor of 1,000 has been removed. The entire Rule of Thumb text has been removed. Section 2.2.4 has been substantially expanded and now includes a worked example of a Target Organ Toxicity Dose example as suggested by the reviewers.
C. That toxicokinetics (e.g., metabolic interactions) can be the determinant of mixtures interactions has been clearly included, see response to charge question 1 and section 1.6 of the document.
D. The approach has been restructured and communicated in a manner to clearly indicate that the decision to proceed with developing a physiologically based pharmacokinetic analysis to mixtures risk should be undertaken on a case bycase basis.

3. Are you aware of any additional information which can be used to improve the present draft report?

Reviewer Response:

A. Examples from the literature, demonstrating the effect of interactions on toxicity. Presentation of the literature on receptor interactions. Mention of other

types of interactions (enzyme induction, non-competitive inhibition, etc.). Equations describing interactions.

B. A good example of a mechanism of interaction that would have very little to do with the proposed RfD-based cumulative analysis is the potentiation of OP toxicity by tieing up serum carboxylesterases. Pretreatment with low toxicity pesticides like fenitrothion and other low toxicity chemicals such as bis-p-nitrophenyl phosphate (BNPP) can potentiate malathion, soman, etc. The LOAEL, NOAEL and RfD for the potentiating agents would be based upon other endpoints that would not reflect their ability to interact with OPs.

References: Chambers, et al., Effects of 3 reputed carboxylesterase inhibitors upon rat serum esterase activity. Neurosci Biobehav Rev 15: 85-88, 1991.

Cohen, Mechanisms of toxicological interactions involving organophosphate insecticides. FAT 4: 315-324, 1984.

EPA Response:
A Other types of interactions have been included, specifically in section 1.6. An example of enzyme induction has not, however, been included, neither has one on receptor binding been included. The report is constructed to guide the risk analyst through the process of justifying and undertaking physiologically based analysis of mixtures and or cumulative risk. As such, more than a few examples are not warranted.

B The information contained in the references suggested by the reviewers has been included in passages inserted into section 1.6. This passage describes OP and AChE interactions in more detail.

4. Are there assumptions or uncertainties being made in this exercise that are not articulated?

Reviewer Response: Use of Hazard Index approach for estimating threshold for metabolic interactions. (although effect threshold is ultimate issue.)

EPA Response: The application of the Hazard Index approach does not and is not intended to address metabolic interactions. Perhaps the reviewer misunderstood something. No change made.

5. Are the figures and tables informative? Would revision of the tables or figures improve the clarity of the report or its conclusions? If so, what are they?

Reviewer Response: Need flow chart of 10 steps and diagram of multiple levels of interactions (PK, PD).
Need table of definitions.

EPA Response: A flow chart of operations has been developed and inserted as Figure 1. It divides the activity into the Initial Assessment, comprising steps 1-5, and the Dosimetry-Based Cumulative Risk Assessment, comprising steps 6-10.

6. Are there important publications missing from the reference section?

Reviewer Response: Mehendale et al. (effects on repair of injury). Swartout et al. (combining HQs). Target organ toxicity doses (EPA/ATSDR publication). Krishnan et al. (BTEX). Andersen et al. (TCE/DCE). Bull et al. (TCA/DCA). Chambers et al., Pope et al. (OP mixture studies). ATSDR interaction profiles. Rider et al. (human OP studies). Paul Price (P3M). Herzberg (MIXTOX database).

EPA Response:

- The reviewers point out several areas of work which could enhance the depth of the report and/or broaden its treatment of uncertainty. The report has been revised to include an example from Mehendale (Tissue repair: an important determinant of final outcome of toxicant-induced injury. Toxicol Pathol. 33:41-51, 2005) on pre-exposure and its effect on stimulating tissue repair near the end of section 1.6.
- Swartout's reference on probabilistic approaches to developing reference doses (A probabilistic framework for the reference dose (probabilistic RfD) Risk Anal. 18:271-82, 1998) was considered but not included due to its indirect relationship to pharmacokinetics and the assessment of exposure.
- A complete and substantial treatment of Target Organ Toxicity Doses has been incorporated in section 2.2.4. References from the US EPa and from Mumtaz have been cited.
- The reviewer suggests inclusion of a publication by Krishnan and colleagues on BTEX. Instead, the report has been revised to include a reference from Dobrev and colleagues that addresses the same issue (thresholds for metabolic interactions) as does the Krishnan paper. This example can be found a couple of pages into section 1.6. The Bull publication on TCA/DCA could be one of several that, en masse, conclude that dichloroacetic acid is a metabolite of trichloroacetic acid and that the liver tumors produced by DCA and TCA are phenotypically different, when cell membrane proteins are evaluated. Bull and colleagues concluded that, when administered separately, TCA and DCA promoted the outgrowth of different subpopulations of spontaneously-arising tumors in rodents. A complicating factor for estimating internal doses of DCA is that administration of DCA at high doses tends to inhibit its own metabolism, prolonging biological residence time. It is not immediately clear how the inclusion of these points would improve the manner in which the document informs the risk assessor in choosing whether and how to implement PBPK modeling to refine mixtures or cumulative risk assessment.
- A PubMed search revealed no returns for organophosphate mixture studies published by Jan Chambers or by Carey Pope.

- The reviewer suggests that the reference list should include some Interactions Profiles developed by the ATSDR. A reference to that database has been added to section 2.2.1.
- Extensive searching for Rider and human organophosphate returns was fruitless. However, a chapter in the PhD dissertation (2005) for Cynthia V Rider demonstrates the development and application of a mathematical model for inhibition of a detoxicating enzyme in a mixtures toxicity context:

  > An Integrated Addition and Interaction (IAI) model of mixture toxicity was constructed and validated using a ternary mixture of organophosphates (malathion and parathion) and the P450 inhibitor piperonyl butoxide. Individual chemical concentration-response parameters and binary interaction data were used in the model. Modeled data was compared to experimentally derived data from *Daphnia magna* acute toxicity assays. The IAI model provided a good fit to the data. Results indicated that toxicokinetic interactions could be quantified and incorporated into mixture toxicity models.
  > http://www.lib.ncsu.edu/theses/available/etd-01022006-223335/unrestricted/etd.pdf

  However, this is not a human data set, and the example is the one for piperonyl butoxide, already incorporated into Section 1.6. No change was made.

- The reviewer has suggested that the document could be improved by including reference to the work done by Paul Price and collaborators, especially that from the P3M database. This database contains measured variability in physiological parameters in humans. Because variation in these parameters may influence internal dosimetry, they can influence risk. Application of the data in the database would improve the estimates of variability in internal dosimetry for a given chemical, but because variability in a given direction for one parameter can increase dosimetry for one compound while decreasing dosimetry for another compound, it is not clear that inclusion of this work would increase the document's treatment of internal dosimetry as a modulator of risk, except when a PBPK model has already been developed for the chemical(s) of interest. As such, this work (abstract from the 2003 meeting of the Society for Risk Analysis below) has not been included in the revisions to the report.

  > **Modeling Inter-individual Variation in Physiological Factors Used in PBPK Models of Humans, by PS Price et al. SRA, 2003,**
  > Modeling interindividual variation in internal dose in humans using PBPK models requires data on the variation in the physiological parameters across the population of interest. These data should also capture the correlations between the values in each person. In this project, we developed a tool to provide such data and its correlations. The tool provides a source of data for human physiological parameters where 1) the parameter values for an individual are correlated with one another, and 2) values of parameters vary according to interindividual variation in the general population, by gender, race, and age. The parameters investigated in this project include: 1) volumes of selected organs and tissues; 2) blood flows for the organs and tissues; and 3) the total cardiac

output under resting conditions and average daily inhalation rates. These parameters are expressed as records of correlated values for the approximately 30,000 individuals evaluated in the NHANES III survey. Software was developed that allows records to be retrieved randomly from the database with specification of constraints on, age, sex, and ethnicity. The [P3M] database and accompanying software together provide a convenient tool for parameterization of human PBPK models for the study of interindividual variation. In addition, the data provides a useful information on the variation in physiological parameters in adults and children. This work was funded by the American Chemistry Council.

- The reviewer also suggested the MixTox database developed by Hertzberg and collaborators. While this database was useful, especially in assessing the likelihood of interactions in binary mixtures, it has not been maintained and is no longer publicly available.

7. What additional information would you like to see presented?

Reviewer Response:
A. Receptor theory (e.g., from textbook by Pratt and Taylor). Mechanism vs. mode of action and implications for interactions (AChE reversible inhibition vs. aging vs. ion channel disruption. The document over-simplifies the nature of OP inhibition of AChE, which can be either reversible or irreversible (aging). Complex mixture issues (DBPs, gasoline).

B. Some discussion of time element (variation in exposure) and linkage to exposure modeling.

C. How to identify potential exposures is not covered. Need indication of completed exposure pathway for mixture to define need for CAG.

D. Need clearer criteria for step 5 cutpoint. (availability of data?, evidence of interaction?). Just common metabolism enzymes is not the only criteria.

EPA Response:
A.

- The document will not be revised to include receptor theory. The reviewer seems to indicate that the document would benefit from inclusion of the distinction between mechanism and mode of action. The basis for deciding the grouping of chemicals for CRA is the Common mechanism Group (CMG) per US EPA guidance. Note that this distinction is made at the level of "Mechanism", not Mode. A key point underlying this comment is perhaps sentiment that critical toxicologic interactions can be based on effects that are not directly involved in the mechanism of action, or are based on events included in the mode, but not the mechanism. Such an interaction based on the former is exemplified by piperonyl butoxide, as can now be found in Section 1.6. The opening passages in section 1.6 have also been revised to include a distinction between Mechanism and Mode and a caveat about how

focusing only on mechanism can result in an overlooking of important bases for chemical interactions.

- The document has been revised to include a more technical treatment of OP interactions, specifically to include binding half-life and aging of enzyme complexes. This can be found in a separate paragraph near the end of section 2.2.4.

- The reviewer indicates that complex mixtures such as gasoline and drinking water disinfection byproducts should be addressed. It is felt that the considerations presented in this document apply equally to simple and to complex mixtures. No revision made.

B. The reviewer indicates that complex mixtures such as gasoline and drinking water disinfection byproducts should be addressed. It is felt that the considerations presented in this document apply equally to simple and to complex mixtures. No revision made.

C. Per the reviewer's suggestion, a passage has been added to the end of section 2.2.2 which communicates the 5 steps identified by ATSDR and references their 2005 guidance.

D. There is not going to be a more clearly made cut-point. The document instead espouses a case by case approach to the decision.

8. Please comment on the overall quality of the report and analysis. What is your overall evaluation of the scientific content, readability and utility of the draft report? Do you have any suggestions relative to structure or content that would improve the quality of this draft report?

Reviewer Response: Excellent explication of mixtures issues within the context of a standard paradigm. The document makes its main point (the importance of considering metabolic interactions) well. The main requirement to improve it is some discussion of other levels of interaction (PD) and examples from the literature of the impact of PK and PD interactions on cumulative risk assessment.

Put reaction network discussion in footnotes or delete.
Tone down language ("wonderful").

"Comprehensive" implies more than just target tissue based. Refer to as "dosimetry-based" instead.

EPA Response:
A. Several additional interaction types and results have been included in the report including metabolic induction, metabolic inhibition, and alterations of biological response (dynamics, induction of tissue repair).

B. Reaction network modeling was mentioned in two places in the draft report. It was retained in the first instance, but was there relegated to a footnote. It was deleted in the second instance.

C. There were several subjective and biased terms included in the external review draft. These sporadic instances of overzealousness have been replaces with more objective terminology.

D. CCRA has been globally changed to Dosimetry-Based Cumulative Risk Assessment (DBCRA).