SAP Report No. 99-06
February 4, 2000

REPORT

FIFRA Scientific Advisory Panel Meeting, December 8-9, 1999, held at the Sheraton Crystal City Hotel and Days Inn Crystal City Hotel, Arlington, Virginia

Sets of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Session I - Characterization and Non-Target Organism Data Requirements for Protein Plant-Pesticides
Session II - Cumulative Risk Assessment Methodology Issues of Pesticide Substances that Have a Common Mechanism of Toxicity

NOTICE

This report has been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). This report has not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, the contents of this report do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The SAP was established under the provisions of FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, to provide advice, information, and recommendations to the EPA Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP) and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the SAP on an ad-hoc basis to assist in reviews conducted by the SAP. Further information about SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Larry Dorsey, SAP Executive Secretary, via e-mail at dorsey.larry@.epa.gov.

TABLE OF CONTENTS

Session I - Characterization and Non-Target Organism Data Requirements for Protein Plant-Pesticides [SAP Report No. 99-06A]
PARTICIPANTS5
INTRODUCTION8
DETAILED RESPONSE TO THE CHARGE12

Session II - Cumulative Risk Assessment Methodology Issues of Pesticide Substances that Have a Common Mechanism of Toxicity [SAP Report No. 99-06B]
PARTICIPANTS 26
INTRODUCTION27
DETAILED RESPONSE TO THE CHARGE32
REFERENCES 47
APPENDIX 49

Notes

SAP Report No. 99-06A, February 4, 2000

REPORT:

FIFRA Scientific Advisory Panel Meeting, December 8, 1999, held at the Sheraton Crystal City Hotel, Arlington, Virginia

Session I - A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Characterization and Non-Target Organism Data Requirements for Protein Plant-Pesticides

Mr. Paul Lewis
Designated Federal Official
FIFRA/Scientific Advisory Panel
Date:_____

Christopher Portier, Ph.D.,
Chair
FIFRA/Scientific Advisory Panel
Date:_____

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
December 8, 1999

SESSION I - Characterization and Non-target Organism Data Requirements for Protein Plant-pesticides

PARTICIPANTS

FIFRA Scientific Advisory Panel Chair
Christopher Portier, Ph.D., NIEHS, Research Triangle Park, NC

FIFRA Scientific Advisory Panel Members
Mary Anna Thrall, D.V.M., Professor, Department of Pathology, College of Veterinary Medicine & Biomedical Sciences, Colorado State University, Fort Collins, Colorado

Food Quality Protection Act Science Review Board Members
George Bruening, Ph.D., Professor, Department of Plant Pathology, University of California Davis, Davis, CA
Arthur Buikema, Jr., Ph.D., Professor, Virginia Polytechnic Institute, Blacksburg, VA
John Fletcher, Ph.D., Professor, University of Oklahoma, Norman, OK
Richard Hellmich, Ph.D., USDA-ARS Corn Insect Research Unit, Insectary Building, Iowa State University, Ames, IA
Barry J. Jacobsen, Ph.D., Professor, Montana State University, Department of Plant Sciences, Bozeman, MT
Rosalind James, Ph.D., USDA Agricultural Research Service, Kika De La Garza Subtropical Agric. Res. Center, Beneficial Insects Research, Weslaco, TX
Mark Sears, Ph.D., University of Guelph, Department of Environmental Biology, Ontario, Canada
Hachiro Shimanuki, Ph.D., USDA-ARS, Bees Research Laboratory, Beltsville, MD
Allison Snow, Ph.D., Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, OH

Designated Federal Official
Mr. Paul Lewis, FIFRA Scientific Advisory Panel, Office of Prevention, Pesticides and Toxic Substances, Environmental Protection Agency, Washington, DC

PUBLIC COMMENTERS

Oral statements were made by:
Ms. Charlotte Arnold, on behalf of the Center for Food Safety
Lincoln Brower, Ph.D., on behalf of Sweet Briar College
Graham Head, Ph.D., on behalf of Monsanto Company
Jane Rissler, Ph.D., on behalf of Union of Concerned Scientists
Demetra Vlachos, Ph.D., on behalf of Novartis Seeds, Inc.
Larry Zeph, Ph.D., on behalf of Pioneer Hi-Bred International

Written statements were received from:
American Crop Protection Association
Monsanto Company
Novartis Seeds, Inc.
Pioneer Hi-Bred International Inc.
Sweet Briar College

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency regarding issues pertaining to the assessment of residential exposure to pesticides. Advance notice of the meeting was published in the *Federal Register* on November 15, 1999. The review was conducted in an open Panel meeting held in Arlington, Virginia, on December 8, 1999. The meeting was chaired by Christopher Portier, Ph.D. Mr. Paul Lewis served as the Designated Federal Official.

The Agency presented proposed data requirements for protein plant-pesticides, including both characterization and effects to non-target organisms. Characterization included the identity of the pesticidal substance and the genetic material necessary for its production along with expression levels, tissue specificity, and plant biology. Discussions included the appropriate non-target organisms for testing, test substances, and conduct of such studies. Janet Andersen, Ph.D. (Office of Pesticide Programs, EPA) provided introductory remarks on the goals and objectives of the session. John Kough, Ph.D. (Office of Pesticide Programs, EPA) and Zigfridas Vaituzis, Ph.D. (Office of Pesticide Programs, EPA) discussed product characterization and effects to non-target organisms, respectively, of protein plant-pesticides.

CHARGE

The specific issues to be addressed by the Panel are keyed to the background document, "Characterization and Non-Target Organism Data Requirements for Protein Plant-Pesticides," memorandum dated November 9, 1999, and are presented as follows:

Issue #1 - Product Characterization

1. Are the presented characterization data requirements adequate to describe the introduced trait for risk assessment purposes?

2. It is well known that expression of an ingredient in a plant as a percentage of fresh weight is subject to gross errors due to the variable water status of plant tissue. To provide a more consistent basis to compare expression levels between tissues or products, is it more appropriate to express protein plant-pesticide levels as a percentage of total protein or as a percentage of dry weight tissue?

Issue #2 - Non Target Organism Data Requirements

3. Is a non-target insect risk assessment based on three selected representative single species and the honey bee adequate? (Considering the improbability of being able to test all of the insects exposed to pesticides? e.g. there are >750 species of butterflies in the USA)

If not:

a. What additional non-target insect species should be tested (taking into consideration the availability of laboratory colonies of the insects)? What criteria should be used to make the selection? (Such as for non-target Lepidoptera)

b. Considering that it is not single species laboratory toxicity which is the basis for the risk assessment, and since it is not practically possible to determine the toxicity to all of the exposed non-target insect species, can definitive higher Tier field scouting data showing the effect of the pesticidal plant on the abundance of non-target species be submitted as support for a request for waiver of some or all of Tier I testing requirements? (or should both single species and field scouting data be required for a risk assessment?)

4. What would be an acceptable number of animals for maximum hazard dose (limit dose) testing vs. the number per replicate for LD50/LC50 determinations? (The current recommendation is 10 per replicate for LD50/LC50 determinations and 30 for maximum hazard dose testing for avian and fish studies. For insect studies the numbers range up to 100 per test group for maximum hazard dose testing).

5. Should OPP extend non-target insect effects testing requirements to include secondary exposure scenarios, like pollen covered milkweed and lupine or intoxicated aphids?

6. Is the maximum hazard dose at 10-100 x the EEC sufficient for non-target insect hazard determination?

7. Are the currently used test duration times adequate? Should they be changed? Currently the longest practical time is required and therefore, for insects, is dependent on how long the species can survive under laboratory conditions (avian - 8 days; fish - 20 days; daphnia - 2 to 21 days; honey bee - 8 to 15 days; earthworm - 14 days; Collembola - 28 days; lady bird beetle 21 days; parasitic wasp- 15 days; green lacewing larvae - 7 to 9 days). Alternatively, is a conventional acute, short duration study acceptable?

8. Current plant-pesticide environmental expression and fate studies (the concentration and degradation rates of the proteins in soil and plant residues) are required to be submitted as Tier I data with the registration application, while the guidelines for other biopesticides list them as Tier II data. Should we continue to require soil degradation data in Tier I for plant-pesticides?

9. The overriding consideration in the case of plant-pesticides is to administer a sufficiently high dose of the test material to obtain a realistic measure of intrinsic toxicity of the test substance to non-target species. Plant tissue toxin levels are often too low to detect toxicity in insects when pure toxin testing shows a hazard. What are the Panel's recommendations to the following test dosing for insect testing?

(a) Dose with transgenic plant tissue whenever possible

(b) Dose with purified (bacterial) protein

(c) Dose with transgenic plant tissue/pure toxin combination to detect possible plant tissue secondary effects

(d) Dose with both the (b) and (c) regimens above

10. Should OPP continue requiring earthworm and Collembola testing for protein plant-pesticides?*

* It was originally thought that since long-term exposure of soil organisms to plant-pesticides is possible when crop residues are incorporated or left upon the soil surface, EPA would require studies evaluating effects upon the representative soil organisms Collembola and earthworms. (This testing was not required by the Agency for registration of conventional pesticides or spray *Bacillus thuringiensis* products). However, there is no evidence that Bt toxins are exuded into the soil by Bt crops, or that Bt proteins are in a form that cannot be readily degraded by soil biota. One of EPA's reasons for requiring the non-target soil invertebrate tests was the concern that adverse effects on these species would cause a build up of plant detritus in cotton fields. However, in reconsideration, EPA discovered that the long term soil use of highly toxic chemical insecticides, such as aldicarb, terbufos, phorate and carbofuran, which have long term effects on soil invertebrate species, has not resulted in the build-up of plant detritus in soils based upon available information on current routine agronomic practices. Some of these materials had half-lives of 10 or more years. Thus protein plant-pesticide crops, which are expected to have less impact on these species than the highly toxic chemical pesticides, should not result in any increased build up of plant detritus. Supporting this conclusion are data which indicate that Bt toxin production in plant-pesticides ceases at plant senescence in the majority of registered Bt corn crops, allowing some time for protein degradation prior to harvest. Additionally, the environmental fate data indicate that for currently registered Bt corn crops only <1 to 90 grams of Bt protein per acre would enter the soil as a result of post harvest incorporation of Bt plants. Since proteins are known to degrade rapidly in the soil (and in-house and published data show a soil half-life of approximately 5 days), the potential for significant soil buildup and hazard to non-target soil organisms is not anticipated from the growing of crops containing protein plant-pesticides.

PANEL RECOMMENDATION

The Panel commended the Agency for the manner in which the meeting was conducted. Agency presentations were well prepared and organized, and the discussions were well led. The Panel did recommend that the Agency consider a few additional data requirements.

The Panel generally agreed with the Agency on the current data requirements for product characterization, except that an annotated map of the vector plasmid should be a general requirement. A DNA construct that satisfied registration requirements in one crop may be part of the application for use in another crop plant. The applicant may use citation from the first registration to compensate for part or all of what otherwise would be phenotype testing

requirements. Under these circumstances, the Agency should require detailed analysis (copy number, possibility of in-frame fusion on insert to host sequences) of the DNA construction as it is inserted in the genome of the new crop, to establish substantial equivalence with the construction in the registered crop plant.

Expression levels of pesticidal proteins should be reported as a percent of total plant protein or total plant dry mass and not as a percent of fresh plant weight. It is also important for the Agency to be given information on the effects that plant stress and phenology have on protein expression levels. In particular, the Agency needs to know what the maximum expression levels might be.

The Panel also agreed that the current non-target insect data requirements were not adequate, mainly in that more species should be considered for testing, although a specific species list was not recommended. Determining which nontarget species to test should depend on the pesticidal plant and how it is to be cultured. The Panel did agree that the Agency should consider adding insect species that are important for biodiversity but not necessarily insect predators or parasitoids. Such test species might include aquatic insects and non-target relatives of the target pest (e.g., non-target Lepidoptera or Chrysomelidae).

Several times during discussions, it was noted that more field scouting data should be required by the Agency, at least until we better understand the ways in which non-target organisms are exposed to transgenic plant toxins in the field, and how toxicity affects non-targets at the population level. Field scouting data could provide valuable information for risk assessment in light of uncertainties about how to expose non-target insects in the laboratory. At the same time, detailed protocols and guidelines are needed because the credibility of field data is dependent on experiments that are well designed and carefully conducted. The Panel concluded that bioaccumulation is not expected to occur with transgenic proteins because biodegradation mechanisms for proteins are ubiquitous. However, there was concern expressed regarding gene transfer from the transgenic plants to similar species which could possibly lead to greater-than-expected expression of the protein in the environment. This "biomultiplication" needs careful study by the Agency but is beyond the purview of this review.

Current Agency requirements for hazard dose levels, test duration times, and replicate size are reasonable, although the Panel did have some specific recommendations for improvement. It was also recommended that a positive response to the hazard dose should trigger Tier 3 testing requirements as well as dose response tests. The Panel also supported using environmental fate studies that are based on the Agency Pesticide Testing Guidelines. Toxicity testing against Collembola and earthworms are probably not good indicators of the potential effect of the pesticidal protein on decomposition, but these organisms do play an important role in other environmental processes and so should not be discontinued.

DETAILED RESPONSE TO THE CHARGE

Issue #1 - Product Characterization

1. Are the presented characterization data requirements adequate to describe the introduced trait for risk assessment purposes?

The Panel agreed that the characterization data requirements as presented in the Agency's background document seem generally to be adequate for the purposes of risk assessment. However, there should be a relationship between product characterization data and non-target organism data in the proposed requirements. The adequacy of presented characterization data depends in part on the extent and type of phenotype testing that is anticipated for non-target organism and other data. Data citation may be used as a compensation for some phenotype testing. When a recombinant DNA construction that is in an already registered transgenic crop plant is substantially the same as a DNA construction used to create the transgene in another crop species or line that is a candidate for registration, requirements for phenotype testing may be reduced or waived. Where data citation reduces requirements for phenotype testing, the Agency should consider a requirement that substantial equivalence be demonstrated between the DNA sequence insertion in the reference plant line and the DNA sequence insertion in the line that is proposed for registration. Considerations include copy number of the inserted sequence and the possibility of a protein fusion between fragmentary inserted donor sequences and recipient sequences (restriction fragment analysis may be sufficient for this purpose).

The document "Appendix I: Molecular Genetic Characterization Data" of the Canada-U.S. Bilateral on Agricultural Biotechnology suggests that an annotated map (Table 1 and Figure 1 of the document) of the plasmid, from which the transgene is to be derived, be part of the submitted data. An annotated plasmid map should be a requirement of the submission for registration.

To better define data requirements for introduced traits, more information is needed about potential recipient organisms generally and about the extent to which genetically modified plants cross-pollinate with wild relatives. Relying on the OECD Consensus Documents on the biology of recipient organisms is not sufficient. These documents are useful, but they are not always up-to-date, especially with regard to information about weeds that can hybridize with crops. In addition, the OECD reports do not provide enough information about the ecology of weedy, wild relatives. For example, it is important to know more about the distribution and abundance of weedy relatives in both agricultural and unmanaged areas. In some cases, the Agency will need information that is not available in the scientific literature in order to evaluate questions about the consequences of crop-to-wild gene flow.

2. It is well known that expression of an ingredient in a plant as a percentage of fresh weight is subject to gross errors due to the variable water status of plant tissue. To provide a more consistent basis to compare expression levels between tissues or products, is it more

appropriate to express protein plant-pesticide levels as a percentage of total protein or as a percentage of dry weight tissue?

Variability in plant water status can have dramatic effects on measurement of any plant component since water is typically in the 85-90% range of plant tissue composition and this is subject to rapid fluctuation and change both in intact plants and excised organs. Expression as percentage of total protein can be affected by extraction procedures and will vary greatly by plant tissue and life-stage of the plant. For these reasons, tissue concentrations of plant-pesticide proteins based on fresh weight measurements will very likely lead to error due to poor reproducibility under different environmental conditions, harvest methods, and investigator judgements. Therefore, it would appear that expressions of plant-pesticide proteins as a percentage of either total protein or dry weight will improve the quality and reliability of protein expression data.

Of perhaps greater importance to the quantity of protein produced (expression) is the physiological condition of plants producing pesticidal proteins in regard to light quality and intensity, water and nutrient availability and presence of other stresses (e.g. heat, pests, diseases, etc.). Field produced plants with minimal stress should be used whenever possible. Whatever growth conditions are used, they should be defined and data should address protein produced under different conditions. An additional concern in arriving at accurate pesticide-protein concentrations is to determine whether or not the concentration changes during the development of a tissue and/or organ. It is also necessary to know how different plant stressors influence synthesis and degradation of the pesticide-protein. For example, during a period of temporary drought (a common occurrence in many parts of the U.S.) when leaves experience water stress, does the newly introduced gene continue to be expressed while other gene expression is shut down? How does water stress influence the turnover of the pesticide-protein versus proteins in general? The concentration of the pesticide-protein in water-stressed leaf tissue may be of great importance in making accurate ecological risk assessment. Ideally, the registrant should describe the conditions where the maximum amount of pesticidal protein is produced.

It is critical that these data be available for different plant tissues at appropriate life stages and through senescence so that appropriate toxicology testing protocols can be established. The data on percentage of total plant protein may also be useful in setting dosage for toxicology tests. The decline in total protein of a leaf during senescence is primarily due to the loss of ribulosebisphosphate carboxylase from the chloroplast. If the pesticide protein is not degraded or perhaps is even synthesized during early stages of senescence, then the ratio (%) of pesticide protein to total protein could show a sharp increase while the amount of pesticide protein per unit of leaf material (consumed by grazer) would be unchanged. This potential erroneous information can be avoided by using dry weights to calculate the expression concentrations since total dry weights are largely dependent on the weight of cell wall components that are not degraded during senescence. It is critical that whatever method is accepted that it is harmonized with other federal or state agencies and countries.

Another Panel member disagreed commenting that the determination of appropriate methods for expressing protein plant-pesticide levels should take into account the use that is to be made of the data. If protein levels are to be entered into equations that also include bioassay data, the uncertainty of the outcome may depend far more on the bioassay data than on the chemical data for protein level. In this situation, there should be an option of using plant-pesticidal protein levels expressed as a percentage of total protein (as is common in research on transgene expression). Plant pesticidal protein levels may be converted, if necessary, to a dry weight basis by applying values from tables of protein, as percentage of dry weight, in various plant materials (providing such tables exist).

Issue # 2 - Non-target Organism Data Requirements

3. Is a non-target insect risk assessment based on three selected representative single species and the honey bee adequate? (Considering the improbability of being able to test all of the insects exposed to pesticides? e.g. there are >750 species of butterflies in the USA)

If not:

a. What additional non-target insect species should be tested (taking into consideration the availability of laboratory colonies of the insects)? What criteria should be used to make the selection? (Such as for non-target Lepidoptera)

The consensus of the Panel was that the Agency might need to expand their list of non-target organisms to include species that are shown to be important components of the agroecosystem of the crop plant. This list could include organisms that are important for biodiversity. The selective nature of plant protein toxins to nontarget organisms and the varied extent of exposure of the chosen organisms requires a different paradigm for evaluation than that used for microbial pesticides. The choice of predaceous and parasitic insects is reasonable, but some consideration of the particular species should be given. Many of these insects might not receive any toxin exposure in the field, thus it is important to develop information on the potential for predaceous and parasitic insects to ingest plant proteins by sap, nectar, pollen, or through feeding on the host. This information could then be used to select test organisms, case by case, based on the potential for exposure to plant protein toxins and with due consideration to the potential for rearing and experimental procedures. One Panel member suggested that an aquatic insect should be added to the species list. Other Panel members disagreed with this recommendation, but agreed that under some circumstances the evaluation of an aquatic insect might be appropriate, e.g. for transgenic rice or where the plant protein might be likely to enter waterways.

In summary, nontarget insects should be selected based on their having an ecological association with the crop plant or target pest. The determination of which non-target organisms to test should be done on a case by case basis for each plant construct taking into consideration:

1) A thorough understanding of the biology of the transgenic crop and the environment in which it will be grown, in order to determine the means by which the nontarget organism might be exposed to the toxin and how the environment may influence exposure and susceptibility.

2) Current scientific knowledge and expertise regarding the ecological interactions that occur between the crop plant and other organisms.

3) The means and probability (distribution) of exposure through pollen, plant residues, root or other organ exudates.

4) Nontarget insects that are likely to be susceptible to the toxin because they are phylogenetically related to the target pest.

Insects that are phylogenetically related to the target pest may be more likely to be susceptible to the plant pesticide than other organisms because many of the pesticidal proteins used in transgenic plants are highly selective toxins. Registrants should be required to carefully consider the potential effects of the pesticidal plant on non-target organisms that are related to the target pest. Hazard should be evaluated if a relative (e.g. same Order) of the target pest is relatively abundant in the field environment of the crop plant, and has a high probability of coming into contact with the plant protein. This contact should be by ingestion if the toxin only acts through ingestion. It is most ideal if the actual non-target in question could be tested. Furthermore, one might not be able to obtain a sufficient number of individuals to conduct bioassays with an acceptable sample size and replicate number, that is, the non-target might be an endangered species or it might not be easily reared in the laboratory. In such cases, test species that are closely related to the non-target but that can be easily reared and are not endangered, may be substituted.

If the registrant can show that no close relative of the target pest is placed at hazard when the crop is deployed, then the requirement for this nontarget impact can be waived.

b. Considering that it is not single species laboratory toxicity which is the basis for the risk assessment, and since it is not practically possible to determine the toxicity to all of the exposed non-target insect species, can definitive higher Tier field scouting data showing the effect of the pesticidal plant on the abundance of non-target species be submitted as support for a request for waiver of some or all of Tier I testing requirements? (or should both single species and field scouting data be required for a risk assessment?)

Field scouting is an important tool to risk assessment, but should not replace Tier 1 testing. Only a limited number of species can be tested in laboratory bioassays, but field studies can be used to detail the impacts on species appropriate to the plant pesticide being tested and in a manner that is relevant to determining ecological impacts. It is important that the conclusions drawn from field studies be scientifically sound and not just correlative and that it reflect actual

exposure to the plant pesticide. The agricultural production system is a massive disruption in itself. It is critical that whatever tests are used be appropriate to the plant protein in question and the life stage of particular indicator species involved. Since ecological effects are critical to safety issues addressed by the Agency proposed rules, it would appear that field studies be included in the decision packet. This must address the environments where the genetically modified plant is to be grown. While the Agency and registrants need predictability in data and testing requirements, flexibility is needed because one set of tests is not appropriate for all potential plant proteins to be considered. It appears that the Agency has provided the needed flexibility.

There is great merit in using field evaluations whenever possible, because of difficulties in knowing how surrogate organisms represent communities of organisms and the difficulty in handling some organisms that may prove to be good surrogates. However, the value of field evaluations and their credibility is directly tied to implementation of well designed and carefully conducted test protocols or strategies with defined endpoints to be measured with interpretation methods worked out in advance. The Agency should move as rapidly as possible to develop field-testing protocols and implement them in their data collection and risk assessment evaluations.

The key issues are: the appropriate control for field scouting studies and whether or not field border areas should be included. If field testing is to be required, a set of protocols should be developed. These protocols should include characterization of border areas if they are to be included in the field scouting. The size of the border area needed can be defined by the characteristics of the plant protein and then by the tissues in which it is expressed (e.g., pollen). Since these tests require large amounts of seed, consideration should be given to grant a conditional approval with full approval given after appropriate field scouting data. This could be done concurrently with resistance monitoring requirements.

4. What would be an acceptable number of animals for maximum hazard dose (limit dose) testing vs. the number per replicate for LD50/LC50 determinations? (The current recommendation is 10 per replicate for LD50/LC50 determinations and 30 for maximum hazard dose testing for avian and fish studies. For insect studies the numbers range up to 100 per test group for maximum hazard dose testing).

The Panel could not agree on a particular number of animals for maximum hazard dose testing because it was the consensus of the Panel that the number would depend on the coefficient of variation. For LD50/LC50, a minimum of 30 individuals and five replicates at each dose level should be required and if losses in the untreated control group exceeds 20%, the validity of the test should be questioned.

Based on this position, the consensus of the Panel was that the Agency should provide applicants with detailed recommendations regarding experimental design and data analysis. The Agency should consider how the data will be used and establish an acceptable level of statistical power. Based on these decisions, appropriate tests and sample sizes can be determined. Case in

point, to determine a maximum hazard dose (MDH), the Agency and applicant should agree on a statistical test and level of statistical power, then the applicant can use their experimental coefficient of variation (CV) to determine sample size and replicate number. It is difficult to determine whether the Agency's current recommendations of 10 per replicate for LD50/LC50 determinations, and 30 (bird and fish) and 100 (insect) per replicate for hazard testing are adequate without knowing the CVs and desired levels of power.

One Panel member recommended that the Agency consider the following: five replicates of 10 animals each might be a minimum for each dose used in the LC/LD50 studies. A possible statistical test for the MHD would be the normal Z test (or Chi Square) to determine statistical significance between control and exposed animals. A statistical test of this type may allow exceedance of the 20% mortality of control animals, especially those organisms that are not routinely used in toxicity testing, because the purpose of the MHD is to determine potential effects that might trigger a LC/LD50 study. There are limitations to exceeding the 20% control mortality factor because too much mortality will not allow the detection of a significant effect.

5. Should OPP extend non-target insect effects testing requirements to include secondary exposure scenarios, like pollen covered milkweed and lupine or intoxicated aphids?

In view of recent publications that indicate secondary exposure may pose ecological risk to certain non-target insects, it is imperative that the Agency assume the responsibility to conduct appropriate ecological risk assessment regarding this controversial issue. However, since no test protocols are in place, the Agency is confronted with the difficult task of determining how to obtain high quality, reliable data to use in risk assessment analyses of secondary exposures. The Panel found it difficult to identify an EEC or generally appropriate means for testing the possible effects of secondary exposure.

For most non-target insects, exposure to pesticidal plant-proteins, if it occurs, likely will be by an indirect route, because non-target insects usually do not feed directly on plant tissue. The definitions of such terms as primary exposure versus secondary exposure, or secondary effect versus secondary trophic effect, remain unsettled. Secondary exposure may occur, for example, when predatory insects prey on target pests that have consumed pesticidal plant-protein. Is consumption of pollen by a non-target insect then to be considered secondary exposure or primary exposure?

Any requirement for testing secondary exposures should be based on a reasonable expectation that a plausible and significant indirect effect can occur. Additionally, a large fraction of the non-target insect population within the confines of the field, or a significant fraction of non-target insects in a large geographical area, must be deemed possibly to be at risk. Most scenarios for secondary exposure are not very compelling because of the low concentration of active ingredient likely to be delivered to non-target insects or the small fraction of the non-target insect population likely to be affected. The half-life of a pesticidal protein also is expected to be short. Therefore, the Panel suggests that food chain effects are not likely to be significant.

Mechanisms of adverse effects not involving the expressed pesticidal protein directly are also conceivable, though unlikely. A pesticidal plant-protein could be biochemically altered so as to make it more toxic to a non-target predator than it was in the plant or in the target insect. Conceivably, alterations in plant or target insect metabolism could also generate an entity that is toxic to non-target insects. These considerations make it difficult to design an economical, reliable, Tier I-type of test for effects of secondary exposure.

Thus, additional research is needed on the various possible effects of plant pesticidal proteins on non-target insects. However, where there is no plausible mechanism for significant exposure of non-target insects or no compelling scenario for significant damage to non-target insect populations, registration of crop plants expressing pesticidal proteins should continue. Nevertheless, it will be prudent for the Agency to require registrants to collect scouting data on non-target insects at a few diverse sites over the range in which the commercialized transgenic crop is planted and for a few growing seasons. Although a scouting requirement rarely has been imposed, scouting may be the most reasonable and economical approach to assessing possible secondary effects on non-target insects, given the difficulties in designing appropriate laboratory tests. Field monitoring during the initial commercial introduction of transgenic plant lines will help determine the extent of any effects at the population level. Current regulations for transgenic plants that express Bt-toxins require that a proportion of the crop be planted in non-Bt-expressing varieties to serve as a refuge for slowing development of Bt-resistant target pests. Such refuges could also serve as controls for scouting data collected to determine the effects of the pesticidal plant-protein on non-target insects.

6. Is the maximum hazard dose at 10-100 x the EEC sufficient for non-target insect hazard determination?

In this case, the consensus of the Panel was the higher the maximum dose the better. However, the Panel recognizes that it is not always practical to use the 100 x level. In addition, the hazard dose should be used as a trigger for Tier 3 test.

An appropriate consideration across toxicological studies is the establishment of a 10 to 100 fold safely factor. Foilar concentrations are used to evaluate microbial insecticides where exposure and toxic effects are more likely and where the system provides sufficient flexibility to determine the hazards posed by those toxins. The maximum hazard dose presented by protein toxins in crop plants is often calculated on the greatest concentration posed by the entire plant residue and not a specific tissue or plant substance. It is appropriate that the regulatory process determine the potential for exposure and define the method of exposure for each non-target to be tested before setting the maximum effect level. Under this scenario, the maximum hazard dose should remain at the current accepted level or be reduced to 10x of the currently calculated value for EEC. Statistical procedures should be clearly stated that would trigger the initiation of further tests for LD50 studies or higher tier level studies as discussed in Question 5.

7. Are the currently used test duration times adequate? Should they be changed?

Currently the longest practical time is required and therefore, for insects, is dependent on how long the species can survive under laboratory conditions (avian - 8 days; fish - 20 days; daphnia - 2 to 21 days; honey bee - 8 to 15 days; earthworm - 14 days; Collembola - 28 days; lady bird beetle 21 days; parasitic wasp- 15 days; green lacewing larvae - 7 to 9 days). Alternatively, is a conventional acute, short duration study acceptable?

The consensus was that the times stated in the Agency's background document were appropriate except that the daphnia test should be longer than 48 hours. Some of the other tests could be shortened if necessary, as long as they last at least 7-14 days. An exposure of five days is minimal. In specific situations such as testing brood of honey bees, a longer exposure period will be required.

In any event, test duration times should not be shortened to the times used in standard toxicity tests because the exposure model is different and the pesticidal plant-proteins are usually slower acting than most chemical pesticides. With chemical applications, non-targets are usually exposed to a spray application that decays relatively rapidly; but with plant-pesticides, the toxin is produced by the plant for a long period of time. In light of the fact that it can take 3-5 days for Bt to act on target pests, conventional, acute, short-duration tests are not going to be adequate to test non-target effects for many transgenic plant-pesticides. However, if tests are too long in duration, control mortalities can be high and also make the test inaccurate. A chronic exposure test of 7-10 days could be adequate for many insects. However, a longer test would be better if control mortalities are low (<25%). Longer studies are necessary for measuring sub-lethal effects such as development time, reproductive effects, etc.

Longer tests have several advantages:

1. results in greater ecological relevancy, especially for plants that continuously produce pesticides.

2. allows use of animals that are not routinely utilized in toxicity testing. The guideline of 20% control mortality provides some grace in conducting studies with a range of non-target organisms.

3. provides for delayed toxicity and potential bioaccumulation of a plant-pesticide.

4. allows for assessment of long term effects on growth and reproduction, especially for daphnia.

The length of exposure should be related to the life cycle of the plant species and/or the length of time the plant-pesticide is produced by the plant or plant tissue.

8. Current plant-pesticide environmental expression and fate studies (the concentration and degradation rates of the proteins in soil and plant residues) are required to be submitted as Tier I data with the registration application, while the guidelines for other biopesticides list them as Tier II data. Should we continue to require soil degradation data in Tier I for plant-pesticides?

The consensus of the Panel was to retain the requirement for data on soil degradation as a Tier 1 requirement as a case by case basis. Since fate studies are already part of Tier I studies in risk assessment, the guidelines for traditional pesticides should apply here. Persistence of a plant-pesticide can be used to red-flag other potential effects that are not apparent from the biological toxicity studies requested in Tier I. It is conceivable that fate data may be useful on a case by case basis to shorten some of the exposure studies recommended for Tier I since registrants routinely conduct fate studies early in their assessment process and should have this information available. Soil degradation data for Tier I plant-pesticides should be required especially if the pesticide is exuded from the plant. Exposure of a plant-pesticide through the soil is different from the consumption of plant tissues used in the other Tier I studies. The demonstrated presence of plant pesticides in the soil should trigger tests with other organisms.

9. The overriding consideration in the case of plant-pesticides is to administer a sufficiently high dose of the test material to obtain a realistic measure of intrinsic toxicity of the test substance to non-target species. Plant tissue toxin levels are often too low to detect toxicity in insects when pure toxin testing shows a hazard. What are the Panel's recommendations to the following test dosing for insect testing?

(a) Dose with transgenic plant tissue whenever possible

(b) Dose with purified (bacterial) protein

(c) Dose with transgenic plant tissue/pure toxin combination to detect possible plant tissue secondary effects

(d) Dose with both the (b) and (c) regimens above

The Panel agrees with the Agency's assessment that when testing non-target species, administering a high dose of test material is useful to obtain a measure of intrinsic toxicity. The Panel recommends that, whenever possible, the dose should be administered with transgenic plant tissue (choice a). The Panel recognizes that at times that this is not possible; therefore, a treatment with high concentrations of pure protein (choice b), or a treatment where the plant tissue test substance is spiked with pure pesticidal protein (choice c) is useful. As a second choice, the Panel recommends dosing with purified (bacterial) protein. Although, with some species a sequential approach could be useful, (e.g., plant material followed by high protein dosing). The Panel highly recommends that when purified protein is used, the similarity or equivalence of this alternate source to that expressed in the plant be established. The Panel acknowledges that isolating purified protein from the plants is difficult and has limited yield, so an *E. coli*, yeast, or other expression system should be considered for producing the protein.

One Panel member had a different position. The material used for dosing should be decided on a case by case basis depending on the plant, the level of gene expression, and the nature of the pesticide compound. In those cases where an expressed gene leads to a protein that changes the metabolism of the tissue, there is a possibility that elevated levels of toxic intermediates and/or end-products may accumulate. Conducting at least some of the dosing tests with altered plant tissue is a means of identifying toxicity that may arise from unexpected

metabolic events.

10. Should OPP continue requiring earthworm and Collembola testing for protein plant-pesticides?*

*** It was originally thought that since long-term exposure of soil organisms to plant-pesticides is possible when crop residues are incorporated or left upon the soil surface, EPA would require studies evaluating effects upon the representative soil organisms Collembola and earthworms. (This testing was not required by the Agency for registration of conventional pesticides or spray *Bacillus thuringiensis* products.) However there is no evidence that Bt toxins are exuded into the soil by Bt crops, or that Bt proteins are in a form that cannot be readily degraded by soil biota. One of EPA's reasons for requiring the non-target soil invertebrate tests was the concern that adverse effects on these species would cause a build up of plant detritus in cotton fields. However, in reconsideration, EPA discovered that the long term soil use of highly toxic chemical insecticides, such as aldicarb, terbufos, phorate and carbofuran, which have long term effects on soil invertebrate species, has not resulted in the build-up of plant detritus in soils based upon available information on current routine agronomic practices. Some of these materials had half-lives of 10 or more years. Thus protein plant-pesticide crops, which are expected to have less impact on these species than the highly toxic chemical pesticides, should not result in any increased build up of plant detritus. Supporting this conclusion are data which indicate that Bt toxin production in plant-pesticides ceases at plant senescence in the majority of registered Bt corn crops, allowing some time for protein degradation prior to harvest. Additionally, the environmental fate data indicate that for currently registered Bt corn crops only <1 to 90 grams of Bt protein per acre would enter the soil as a result of post harvest incorporation of Bt plants. Since proteins are known to degrade rapidly in the soil (and in-house and published data show a soil half-life of approximately 5 days), the potential for significant soil buildup and hazard to non-target soil organisms is not anticipated from the growing of crops containing protein plant-pesticides.**

The majority of the Panel agreed with the Agency in continuing to require both earthworm and Collembola testing for protein plant-pesticides. The Panel agreed that such testing could be waived on a case by case basis depending on the mode of action of the plant pesticide.

Earthworms are important in mixing and aerating soils and act as indicators of good soil health. Earthworms and collembolan act as a food source for many predacious insects in the agricultural field, and for birds. For this reason, the Agency may want to consider keeping earthworms and collembolans as test species of soil invertebrates.

Depending on plant-pesticide, soil type, crop and other factors, it may be appropriate to conduct Collembolan and earthworm tests. For example, if the plant pesticide is a chitin inhibitor or an enzyme that breaks down chitin, insects and earthworms would be appropriate test organisms.

A minority view concluded that microorganisms (i.e. fungi), not earthworms and collembolans, are the main forces for decomposition of crop residues. This is why pesticides that have a large impact on earthworm populations can have low impact on crop residue decomposition rates. Thus, the Agency needs to carefully consider the purpose behind the earthworm and collembolan tests. If the main concern is crop residue buildup, earthworm tests may not be necessary, and the Agency should consider testing the effects of pesticidal plant-proteins on a few key decomposition microbes in the soil. Such tests could be done rapidly in petri dishes in a manner similar to antibiotic-resistance tests routinely done in medical laboratories.

Additional Comments

Both the information provided and the presentation made by the Agency clearly established that the Agency has made timely efforts in the 1970s and 1980s to build a foundation and framework of background information, data, and methods necessary for it to fulfill its regulatory responsibility regarding genetically altered plants. The Agency is to be commended for its proactive efforts in the 1980s when OPP sought input from the general scientific community (i.e., Boyce Thompson Workshop, 1987) and appeared to identify and list key questions of environmental concern. However, it is disappointing and perplexing that the Agency failed to follow through and address the questions its personnel identified in the 1980s. These same questions now appear to be emerging issues (i.e. monarch butterfly and Bt corn).

During the course of the Panel's meeting, there were several discussions regarding the lack of certain test protocols and/or scientific data to justify new or expanded sets of test data from registrants. These discussions suggested that either the Agency has not committed adequate research dollars to this topic in recent years, or the funded research has been misdirected in the sense that it has failed to provide the products necessary for OPP to fulfill its regulatory mission.

Notes

SAP Report No. 99-06B, February 4, 2000

REPORT:

FIFRA Scientific Advisory Panel Meeting, December 9, 1999, held at the Sheraton Crystal City Hotel, Arlington, Virginia

Session II - A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Cumulative Risk Assessment Methodology Issues of Pesticide Substances that have a Common Mechanism of Toxicity

Ms. Laura E. Morris
D.V.M.
Designated Federal Official
FIFRA/Scientific Advisory Panel
Date:_____

Ernest E. McConnell,

Chair
FIFRA/Scientific Advisory Panel
Date:_____

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
December 9, 1999

Session II: Issues Pertaining to Exposure Assessment and Estimating Cumulative Risk

PARTICIPANTS

Chair
Ernest E. McConnell, D.V. M., Raleigh, NC

FQPA Science Review Board Members
John Adgate, Ph.D., Assistant Professor, University of Minnesota School of Public Health, Minneapolis, MN
Amira Eldefrawi, Ph.D., Professor, Department of Pharmacology & Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD
Scott Ferson, Ph.D., Applied Biomathematics, Setauket, NY
Natalie C. Freeman, Ph.D., Adjunct Associate Professor, Robert Wood Johnson School of Medicine, Department of Environmental/Community Medicine, Piscataway, NJ
Dale Hattis, Research Associate Professor, Clark University, Worcester, MA
Edo Pellizzari, Research Triangle Institute, Research Triangle Park, NC
Nu-may Ruby Reed, Ph.D., Staff Toxicologist, California Environmental Protection Agency, Department of Pesticide Regulation, Sacramento, CA
Lorenz Rhomberg, Ph.D., Gradient Corporation, Cambridge, MA
Stephen M. Roberts, Ph.D., Professor and Program Director, University of Florida, Center for Environmental & Human Toxicology, Gainesville, Florida
John Wargo, Ph.D., Associate Professor of Environmental Policy and Risk Analysis, Yale University, New Haven, CN

Designated Federal Official
Ms. Laura E. Morris, FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Environmental Protection Agency, Washington, DC

PUBLIC COMMENTS

Oral statements were received from:
Charles Benbrook, Ph.D., on behalf of the Consumers Union
David Wallinga, M.D., on behalf of Natural Resources Defense Council
Robert Sielken, Ph.D., on behalf of the American Crop Protection Association
Jennifer Phillips, Ph.D., on behalf of the American Crop Protection Association
Charolette Arnold, Esquire, on behalf of the Center for Food Safety

Written statements: None

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP), has completed its review of the set of scientific issues being considered by the Agency regarding the review of issues pertaining to cumulating exposure for pesticides that have a common mechanism of toxicity. Advance public notice of the meeting was published in the Federal Register on November 15, 1999. The review was conducted in an open Panel meeting held in Arlington, VA, on December 9, 1999. The meeting was chaired by Ernest E. McConnell, D.V.M. Ms. Laura Morris served as the Designated Federal Official.

The 1996 Food Quality Protection Act requires the Agency to consider aggregate exposure (i.e., exposure from residential, dietary and tap sources) to a pesticide. The Office of Pesticide Programs (OPP) presented a preliminary draft of proposed guidance on cumulative assessment of pesticide chemicals on September 23, 1999 for review by the FIFRA Scientific Advisory Panel (SAP). The purpose of the review was to seek recommendations from the SAP on the hazard and dose response analyses needed when accumulating risk from exposure to two or more chemicals that share a common mechanism of toxicity. At the September 1999 SAP meeting, OPP indicated that its guidance document is not complete and that the exposure and risk characterization sections of the guidance will be presented at the December 1999 SAP meeting. Toward that end, the Agency presented to the SAP the completed Chapter 4, "Exposure Assessment and Characterization" and Chapter 6, "Estimation and Characterization of Cumulative Risk" for review. In addition, the SAP recommended that the case study presented by the Agency at the September 1999 meeting be developed further by using actual data, including exposure information. Thus, this presentation also provides a revised case study on organophosphorous pesticides for comment by the December SAP.

CHARGE

The specific issues to be addressed by the Panel are keyed to the background documents, ***"Issues Pertaining to Exposure Assessment and Estimating Cumulative Risk (for Conducting Cumulative Risk Assessments on Pesticide Chemicals that Have a Common Mechanism of Toxicity), "Proposed Guidance for Conducting Cumulative Hazard Assessments for Pesticides that Have a Common Mechanism of Toxicity"***, memorandum dated August 31, 1999, and ***"Chapters 4 and 6 for FIFRA Scientific Advisory Panel Review,"*** dated November 10, 1999.

Issue #1: Input Parameters

There are several types of data available for pesticide exposure assessment (e.g., field trial data, monitoring data, percent crop treated, label usage). For the dietary (food) pathway, monitoring data are available from the USDA Pesticide Data Program (PDP). OPP conducts the majority of its drinking water assessments by calculating a screening level value. Similarly, residential assessments are conducted using the draft residential SOPs which also provide a screening level assessment. Thus, given PDP, the assessment of the dietary (food) pathway will,

in many cases, be based on higher quality data than for the residential and drinking water pathways where usually only screening values are available. Because of the different quality of data that will be encountered when conducting a cumulative exposure assessment, the concern is raised that the value and benefit of high quality monitoring data will be lost if combined with extrapolated exposure values from screening models.

> **Question 1a:** Please comment on how this concern could be addressed. For instance, should OPP at this time conduct separate pathway assessments for dietary (food), dietary (drinking water), and residential exposures so as to avoid combining higher quality monitoring data with more limited screening level data?
>
> **Question 1b:** The Panel is asked to comment on whether there are other means of dealing with existing data to reduce the uncertainties about exposure values derived from screening approaches.

Issue #2: Exclusionary Criteria

OPP is proposing that exclusionary criteria be applied to defer consideration of " negligible" sources of risk in a full cumulative risk assessment. OPP believes that this approach will permit a better focus on the more important sources of risk. It will also assist the risk manager in understanding and evaluating sources of risk that may provide the greatest benefit with risk mitigation activities.

> **Question 2:** Please comment on whether the exclusionary criteria discussed in *Chapters 4* and 6 appear to be reasonable. Can the Panel suggest other exclusionary criteria that should be considered?

Issue #3: National and Regional Exposures

The potential for people to encounter overlapping exposures to different pesticides will be influenced by many factors. One important consideration is the geographic effects and seasonal uses of pesticides. Thus, a framework is proposed for assessing different pathways of exposure that are essentially driven by these considerations. OPP believes that the dietary (food) pathway should be approached on both a national and regional scale to account for both national and regional distribution of treated commodities. However, the Office believes that residential and dietary (drinking water) pathways are more appropriately dealt with on a regional or multi-state basis, since there is no single, national source of drinking water; and residential exposures may be driven by regional use patterns.

> **Question 3:** Please comment on whether the concept of developing a series of cumulative assessments on a geographic scale for different pathways is reasonable.

<u>Issue: Case Study</u>

Cumulative risk assessment is at an early stage of development. Furthermore, there is very limited experience in conducting such assessments. Thus, the development of case studies using actual data is critical to refining useful and practical guidance and to identifying future research and testing needs. OPP is taking a stepwise approach to the development of such case studies by starting with simple examples and moving toward more complex situations.

Attached is a case study that uses actual dietary (food) residue data on three pesticides and evaluates only a single pathway/route/duration of exposure. Certain assumptions were made in the case study. In single chemical exposure assessment, for example, nondetects are assumed to be one half the level of detection and composite samples are decomposited. In this case study, for illustrative purposes, nondetects were assumed to be zero, the samples were not decomposited, and surrogate data were not used.

> **Question 4:** Given that an important goal of the cumulative assessment is to reliably determine sources of concern from a multi-chemical exposure, please comment on to what extent it is appropriate to apply standard practices and assumptions used in single chemical assessments.

PANEL RECOMMENDATION

The Panel recommended the following improvements to the approach for cumulative risk assessment as presented in the proposed guidance:

<u>Combining the Use of Data with Varying Quality:</u>

- Clearly document the quality of data and input parameters used in the risk analysis. Quality threshold could be established for data use. Monitoring data, properly accounted for measurement errors, are preferred over screening level inputs.

- Focus on individual-based analysis to ensure capturing the high exposure and sensitive individuals and account for cross-media transfer and "para-occupational" exposures.

- Cumulative risk analysis should retain the resolution of geographic, temporal, and demographic variations while maintaining optimal data usage with respect to the increasing uncertainties associated with lowering of sample size.

- Systematically conduct quantitative sensitivity and uncertainty analysis for both the exposure and the toxicity.

- Uncertainties in data can be reduced or better characterized by (1) comparing sets of

similar data collected from different years and locations, (2) comparing results from screening level analyses with more refined analyses from data-rich cases for selected chemicals and pathways, and (3) maintaining the association between the pathways.

- Develop the process for reassessment as new quality data become available.

Exclusionary Criteria

- The Panel viewed the exclusionary criteria as deferral arguments that realistically allow the Agency to focus on the real issues of major contributors. A broad estimated range of values may suffice to represent the location and uncertainty of low contributing pathways.

- A simple percentile is an inadequate criterion. The exclusion criteria should be accompanied by descriptions that include the considerations for route-specific toxicity, toxicity-weighted absorbed dose, individually based patterns of pathway contribution, and chemical-specific sensitive endpoints. Possible contexts for an exclusion based on the magnitude of contribution are 1) a fraction of the cumulative absorbed dose over the relevant averaging time for individuals, 2) a fraction of an allowable toxicity-weighted exposure (e.g., a fraction of the acceptable total risk) for a low fraction of exposed population, and 3) a certain cumulative lowest fraction of exposure. An accompanying description of “inclusion” based on the exposure patterns of individuals could enhance the description of the “exclusion.”

- The exclusion criteria should accommodate the change in use patterns, avoid omitting collective exposures of many small contributions, and provide for revisiting each exclusion during reassessment or when new data become available.

National and Regional Exposures

- To the extent possible, the assessment should characterize seasonal, geographic, temporal, and demographic variations that capture the full range of cumulative exposures. Knowledge of varying pesticide use patterns and practices is essential for an accurate assessment of cumulative risk.

- The spatial boundaries need to be carefully examined. Difference in "region" applicable to each pesticide can introduce significant bias or inaccuracy. Sampling frames should be well defined in order to avoid the problem of combining multiple data sets collected at widely varying scales of aggregation.

- Case studies should be used to demonstrate the helpful examples given in the draft guidance (examples A-C, pages 13-14) for Common Mechanism Group (CMG) selection and exposure assessment. Thinking through a case of herbicides will elicit several additional issues of defining the regional/local occurrence and broaden the perspective in

developing criteria/guidelines of dealing with such scenarios.

- Displaying the confidence intervals would help to convey the strengths and weaknesses of partitioning databases into smaller components (e.g., population subgroups), as data reliability deteriorates from the reducing sample size. Sampling population weights should be used in the analyses of the dietary data.

- In some cases, subdivision of population groups are best characterized by human activity patterns (e.g., concerns for pet-related pesticides for pet owners).

Case Study Illustration - (Recommendations not already mentioned above**)**

1) Common Assessment Group (CAG):

- Clearly differentiate the criteria for choosing CAG pesticides in terms of common molecular targets or similar toxicity symptoms.

2) Toxicity Assessment

- Replace the NOAEL/LOAEL-based modeling for relative potency with methods that make better use of dose-response information available for the chemicals of interest (i.e., cholinesterase inhibition).

- Expand the discussion on the development and application of the FQPA safety factor and include its illustration in a case study. A safety factor solely based on common mechanism oversimplifies the susceptibility issue due to chemical-specific disposition (i.e., distribution, metabolism, eliminations, etc.), especially pertaining to prenatal and perinatal exposures.

3) Exposure assessment: The case study is overly simplistic. Future case studies should:

- Include pathways other than food, using the technique outlined in the response to Question 1.

- Include distributional exposure analysis for a best possible assessment, showing variability, contribution, and uncertainties for each agent and route.
- Infer statistical distribution for non-detect data points based on the percentage of crop treatment.

- Use decompositing procedures as developed and discussed previously by the SAP to reconstruct distributions of single serving residues.

- Move away from the media-based approach for a single pesticide assessment to an

individual-based analysis.

- Include examples where poor data quality for a pathway drives the overall assessment. Adopting conservative assumptions for when data are lacking could provide incentives to collecting data.

DETAILED RESPONSE TO THE CHARGE

Issue #1: Input Parameters

There are several types of data available for pesticide exposure assessment (e.g., field trial data, monitoring data, percent crop treated, label usage). For the dietary (food) pathway, monitoring data are available from the USDA Pesticide Data Program (PDP). OPP conducts the majority of its drinking water assessments by calculating a screening level value. Similarly, residential assessments are conducted using the draft residential SOPs which also provide a screening level assessment. Thus, given PDP, the assessment of the dietary (food) pathway will, in many cases, be based on higher quality data than for the residential and drinking water pathways where usually only screening values are available. Because of the different quality of data that will be encountered when conducting a cumulative exposure assessment, the concern is raised that the value and benefit of high quality monitoring data will be lost if combined with extrapolated exposure values from screening models.

Question 1a: Please comment on how this concern could be addressed. For instance, should OPP at this time conduct separate pathway assessments for dietary (food), dietary (drinking water), and residential exposures so as to avoid combining higher quality monitoring data with more limited screening level data?

When conducting a cumulative risk analysis that utilizes data from multiple sources of different strength, the data quality should be clearly stated and the input parameters documented regarding quality and reliability of data. If the data are quantitative, they can be used to weigh the results of the assessment using methods analogous to the dose response assessment. Alternatively, qualitative data should be assigned at the point the data enters into the assessment and the quality labels used to assess the overall process in the risk characterization step. Given the lack of data in either event, it will be necessary to develop and use models that propagate uncertainty and distinguish that from variability to perform quantitative estimates of exposure and effectively use existing input parameters.

As new quality data become available where they were absent for pathways leading to exposure, a reassessment should be conducted. The Agency should develop some criteria or process that permits for reassessment, e.g., it might be stated in the initial assessment when and

under what circumstances this might occur.

There will always be considerable variance in the quality of data that are combined to estimate cumulative exposure and risk. Data quality thresholds should be established for each pathway and when these are not met, default assumptions that are conservative (i.e. that assume high exposure) should be used to estimate exposure and risk. Within each pathway where numerous types of data are often combined, quality thresholds should be defined for each type. For example, dietary exposure is dependent upon food intake data and residue data. There are many types of residue data, and these vary in quality by sampling design, sample size, age, and many other factors. Before the Agency adjusts residue estimates for processing effects, the processing studies should be assessed for their quality—which depends upon many factors. A twenty year old study using outdated processing technology should not be used to adjust current residue estimates. If the quality threshold is not reached, the Agency should revert to conservative default assumptions. The effect of such defaults should be tracked using uncertainty analysis (as discussed more extensively below) to make evident the contributions of defaults and to point out places where provision of further data could significantly alter and improve the analyses.

In designing the process of assessing cumulative risk, it would be beneficial to describe more useful categories than merely "dietary-food, drinking water, and residential," since there are many potential exposure scenarios within each of these pathways. The possibility exists that some large and variable exposures for some special populations will most likely drive the upper bounds of the cumulative assessments. In addition, the proposed data sources and methods do not address the issue of cross-media transfer, such as water-to-food transfer (e.g., pesticides picked up in the cooking process) or surface-to-food transfer (e.g., during food preparation, from a contaminated surface, or by a child dropping and then consuming the food). It is likely most useful to frame the analysis in terms of sensitive subpopulations and devote resources to better characterizing the uncertainties for the sensitive subpopulations. Furthermore, the example presented in the document doesn't take into account potential exposures to farm worker children either from spray drift or adult "take home" or "para-occupational" exposures. Use of initial scientific judgement on chemicals and pathways would allow the Agency to devote resources to " worst-first" cases, so that the most egregious potential errors from combining data of varying quality could be avoided.

Available data will always be uncertain reflections of real-world patterns of exposure. Given this conclusion, the Agency should use the best available data and attempt to estimate the magnitude of uncertainty as best it can for each type of data employed in the exposure assessment.

The capacity to test the sensitivity of its exposure estimates to differing assumptions and different data sets should be developed. This should be done in a way that is transparent and graphic. The Agency might consider the construction of a simple spreadsheet model that would identify each factor that contributes to exposure and risk. Such a model could easily allow the

user to change assumptions or data sets, while viewing the effect upon total exposure graphically. This type of interactive sensitivity analysis quickly demonstrates the relative importance of different contributing factors.

The Agency should strive to reduce uncertainty among variables that contribute significantly to cumulative exposure. Scarce research resources should not be expended to reduce uncertainty in a variable that contributes a minuscule proportion of cumulative exposure, unless that slight proportion may carry significant risk due to high chemical potency.

Question 1b: The Panel is asked to comment on whether there are other means of dealing with existing data to reduce the uncertainties about exposure values derived from screening approaches.

One basic goal for conducting a cumulative exposure/risk assessment is to fairly represent what we know and don't know about the combined toxicity that can be expected from multiple compounds with related toxic mechanisms. To achieve this, there is no substitute for a systematic, explicit, and quantitative treatment of multiple sources of both variability and uncertainty. The Agency cannot hope to produce meaningful analyses for decision making of multi-chemical risks from a straightforward combination of (1) off-the-shelf prior assessments of screening level point estimates of high end exposures from multiple routes, (2) more detailed distributional information on exposure from dietary-food routes, and (3) NOAEL/LOAEL point estimates of relative potency. As will be discussed more extensively in comments under Question 2 below, the exemplary case study provided in the draft guidance document fails to provide an analysis that is helpful for decision-making on either needs for new information or possible needs for changing regulatory control efforts because it does not deal systematically and quantitatively with the uncertainties in either the exposure or toxicity inputs to the analysis.

The difficulty of combining data of different quality/uncertainty can be overcome if the uncertainties inherent in different data sources are quantitatively addressed by replacing point estimates derived by traditional procedures with distributions reflecting both (1) any tendencies for systematic "bias" due to built-in conservatism and (2) quantitative estimates of uncertainties. The translation from screening level assessments to appropriate uncertainty distributions can be made in part by comparing the results of screening level assessments of exposure with more thorough and detailed measurements for selected chemicals by similar routes. In this way a limited number of thorough studies of exposure distributions delivered by the routes usually assessed via "screening" methods can help the Agency make more appropriate use of its larger body of screening level assessments without adopting either of the polar-case assumptions implied in the document. These can be either (1) excluding a route of exposure that is suspected to deliver appreciable amounts of the chemical of interest in the cases of some people or (2) adopting the screening level value as if it were a central tendency estimate applicable to the population studied more extensively via the dietary measurements.

In some cases, *accounting for* uncertainty may present a more practical concern than that

of *reducing* uncertainty. The latter generally requires substantive empirical effort beyond the scope of a cumulative risk assessment. The Agency suggests that "qualitative" uncertainty analysis will be used to inform decisions based on the assessments of cumulative risks. The Panel, however, is unified by the opinion that assessments should employ *quantitative* uncertainty analysis. There are several advantages of quantitative uncertainty analysis (QUA).

1) QUA allows a model to combine data of widely disparate quality. Properly deployed, it allows all relevant data to be used in the analysis, even if some do not meet strict data quality guidelines.

2) QUA also permits analysts to complete an assessment before the best possible data are available, and therefore improves the timeliness of the results.

3) QUA allows analysts to use monitoring data in place of screening values. (See below.)

4) QUA automatically produces a statement about the reliability of the results. This analysis provides a context in which the quantitative results should be interpreted and allows people to judge the degree of confidence that should be placed in an assessment.

5) QUA focuses future empirical effort. The parameters that most significantly influence the result can be identified. Uncertainty analysis (and only it) shows where estimates can be tightened to improve the overall assessment process.

The Agency should be much more serious about providing an honest accounting of the reliability of the inputs used in its assessment and, thus, the reliability of the results and conclusions derived from them. Calls for the "best science" should be secondary to this call for an honest science that accounts for and correctly propagates uncertainty through calculations. No point estimate should be used if it lacks an associated statement about its uncertainty.

The Panel agrees that it may be inappropriate and unnecessary to use screening levels as input values in the cumulative assessment. Actual monitoring data could be much more appropriate, but only if the uncertainties associated with these data (including measurement and sampling error) are correctly propagated in the assessment. It would be decidedly wrong to use estimates based on monitoring data without a careful accounting of measurement error. A quantitative uncertainty analysis should be applied at every step in the assessment. For instance, it is rarely reasonable with censored data to replace non-detects with zeros (as was done in the example assessment presented to the Panel). It might make more sense to replace a non-detect with an interval $[0, L_Q]$ where L_Q is the quantification limit. If the uncertainty propagation method is sufficiently good, there will be little consequence for the result. Of course, it may turn out to be the case that properly accounting for all the measurement uncertainties in the input parameters suggests that there is very little surety in the resulting estimate of cumulative exposures and risks. If this is true, however, it would certainly be important to clearly admit this limitation so that unjustified conclusions are not drawn from the assessment results.

There are several approaches that might be used for quantitative uncertainty analysis. Even a comprehensive analysis need not be overly complex. The simplest approach is interval analysis of the plausible *ranges* of input parameters. In interval analysis, point-estimate values of the input parameters are replaced with intervals that are sure to straddle both the natural variability and our scientific uncertainty about a value. An even more useful approach is to use both the intervals and the best available point estimates for each input variable and to propagate these jointly in the analysis. Each input is therefore represented by a set of triple of numbers that together capture its potential range and our best estimate of its value within the range. This approach is vastly preferable to a so-called "worst-case" approach that confounds best estimates and upper bounds together with an unspecified degree of conservativism. These kinds of uncertainty analyses are most appropriate for propagating incertitude (partial ignorance such as measurement error and scientific uncertainty). In some cases, however, variability among individuals in the exposed population will be a more important source of uncertainty in the assessment. In such cases, it would be better to use a probabilistic form of quantitative uncertainty analysis such as Monte Carlo simulation. To use this approach, analysts must estimate the full statistical distributions of the variable input parameters as well as any correlations or other statistical dependencies among these inputs. It may even be helpful to use slightly more elaborate methods such as two-dimensional Monte Carlo simulation, probability bounds analysis, or mixed methods that use intervals within a Monte Carlo simulation. Even the most complex of these methods is still very simple and entirely practical for cumulative risk assessments. Conscientious use of any of these approaches would substantially improve the strategy articulated by the Agency.

Suggestions for reducing uncertainties were made specific to the use of existing database as presented in the case study.

Food Consumption Data: The Agency is currently conducting dietary exposure assessment based on the USDA CSFII 1989-1991 data. This set of data is expected to be replaced by the more recent survey data (i.e., CSFII 1994-1996 data) as they become available to the Agency. Although the two databases cannot be combined to yield a larger sample size due to the differences in survey designs, a comparison of the two may provide valuable information regarding both the uncertainties and variabilities in eating patterns. This is particularly useful for those commodities for which low incidence of consumption was reported on the days of survey (i.e., low consumption days). When estimating the total exposure from all commodities, the deficiencies and uncertainties associated with these commodities with low consumption days are often masked by the inclusion of commodities with high consumption days. A systematic analysis to identify those low-consumption-day commodities will be a very valuable first step in characterizing the underlying uncertainties. It may then be possible to use one set of data to quantitatively estimate the uncertainties associated of the other set.

Pesticide Residue Data: Residue data from a single year were used in the illustrative case study. When residue data from multiple years are available, they should be used to better

characterize the variation that may not be adequately captured in one year of monitoring. Seeking to correlate the fluctuation of residue levels with any major changes in pesticide use pattern would also provide valuable links between the two pieces of data. Other approaches such as estimating the residue levels of non-detect samples, the decompositing of data points for commodities that can be consumed in a single unit are additional means to address the uncertainties inherent in the available database.

Although the PDP data currently used by the Agency is most representative of the residue profiles for the entire U.S., there are a vast number of commodities for which no PDP data are available. Residue data of commodities available nearer to the farm gate (e.g., farmers' markets, u-pick operations) should also be considered. It may be expected that these residue levels would be higher than the samples represented by the PDP and also show a greater geographic and seasonal variations. Statewide or smaller scale residue monitoring data may be useful for characterizing these variations.

Geographic, Temporal, and Demographic Patterns: The draft guidance indicated that dietary exposure can be viewed as the foundation over which water and residential exposures are superimposed. Since the latter two pathways are highly specific to geographic, time and demographic variations, it is important to consider bringing the dietary exposure analysis down to these levels as data would allow. In addition, finer division of age range is also encouraged, particularly for children 1 - 6 years old. A systematic analysis should be conducted to characterize the limitations of the consumption database for the finer division of grouping, identify the associated uncertainties, and articulate the rationale for an optimal level of data aggregation.

Drinking Water and Dietary Exposures: According to the draft guidance, the drinking water exposure pathway may not be included in the assessment of cumulative risk due to the lack of reliable data beyond the screening level estimations. Instead, the evaluation of the drinking water component may be qualitative. As the Panel generally favors a quantitative assessment, the drinking water exposure should be included in a same "dietary" exposure analysis by using the same consumption data (i.e., CSFII), in order to maintain the correlation between the consumptions of food (e.g., consumption of juices) and water. The breakout of forms of water (e.g., non-food water, commercial water) based on the food consumption surveys should be appropriately attributed.

Issue #2: Exclusionary Criteria

OPP is proposing that exclusionary criteria be applied to defer consideration of " negligible" sources of risk in a full cumulative risk assessment. OPP believes that this approach will permit a better focus on the more important sources of risk. It will also assist the risk manager in understanding and evaluating sources of risk that may provide the greatest benefit with risk mitigation activities.

Question 2: Please comment on whether the exclusionary criteria discussed in

***Chapters 4* and 6 appear to be reasonable. Can the Panel suggest other exclusionary criteria that should be considered?**

The exclusion criteria as presented in bullet form on page 4-5 of the draft guidance do not appear to be as fully specified as would be helpful. Further clarification and definition of these criteria are needed. The final criteria should also serve as deferral arguments that realistically allow the Agency to focus on the real issues of major contributors without being encumbered by detail analysis of components that have only marginal contributions.

The first criterion is that one may exclude a particular pathway for a specific chemical that " is likely to contribute less than 1.0% of the total exposure." The Panel raised two questions about this criterion: First, what measure of exposure is to be used in calculating the 1%? It needs to be clearer if this is to be a potential dose or an absorbed dose. The calculation of fractional contribution is likely to differ when a short or a long averaging time is in question, so it must be clear whether the pathway's cumulative exposure (using "cumulative" in the traditional sense of adding up progressive increments to give the total) integrated over the averaging time or a dose rate is intended. It seems most in the spirit of the criterion for the 1% to refer to the fraction of cumulative absorbed dose over the relevant averaging time. This raises the possibility that an exposure pathway may be excludable for some endpoints and not for others.

Since we are dealing with exposure to several agents in a CMG, the issue also arises whether the measure is to be in mass of agent or if it has some sort of allowance for the differential toxicity (via the common mechanism) of the components. A rational application of the criterion would suggest that toxicity-weighted exposures should be used. Otherwise, one may exclude a small exposure (in mg/kg) to a very potent compound and include a larger exposure to a weak agent, even though the first exposure was the larger contributor to cumulative risk.

The second question the Panel raised is whether the 1% is to refer to the whole population, an average individual, or a highly exposed individual (with respect to the pathway in question). It is possible, for instance, that a small number of people have the pathway in question representing 20% of their personal exposure, but that the pathway accounts for less than 1% of the average person's exposure and less than 1% of the total amount of exposure in the whole population of interest. Since there is a need to address individual as well as population risks, it seems appropriate to include exposures that make up a significant fraction of the personal exposure of anyone (or at least almost anyone). Failing to do this would inappropriately exclude high but uncommon exposures from consideration.

A pitfall is that a fraction of a person's exposure from a pathway may be large either because he has a large exposure by that pathway or because he has small exposures by all *other* pathways. For example, the same 1 mg/kg could be 20% of the exposure of a person with a total of 5 mg/kg and only 0.5% of the exposure of a person with 200 mg/kg total. It would seem foolish to exclude the pathway in the latter case and not in the former.

In short, a more specific definition of the 1% criterion is needed. In view of the above

considerations, the Agency may want to consider a criterion based on a percentage of an allowable toxicity-weighted exposure as the exclusion criterion. For carcinogenic agents, this would amount to a fraction of the acceptable total risk, while for noncancer endpoints it would be a fraction of the "risk cup" (using older terminology). It should probably be expressed as the likely exposure not exceeding X% of the allowable exposure in more than Y% of the exposed population, with both X and Y being fairly small percentages.

The Panel noted that 1% is a fairly restrictive cutoff in the sense that only the smallest pathways would be excluded. (This is especially true if it has to be met by most people in a population of varying exposure.) Given uncertainties in exposure assessment, it may be hard to assure that the criterion is met. Moreover, there may be very many minor pathways, each excludable under the 1% rule. Together, these may total to a rather high cumulative exposure, and if they were excluded, the true total would be significantly underestimated. It was suggested that the Agency may want to consider an alternative criterion that the cumulative lowest Z% (say, 5%) of the exposures can be excluded, i.e., once (100-Z)% (e.g., 95%) of total exposure is accounted for, the balance can be excluded.

The Agency's second proposed criterion is that *"a specific pesticide-pathway combination makes a negligible (<1%) contribution…because of limited use or low consumption of the treated commodity*". As noted above, this could exclude an exposure that is a small fraction of exposures with long averaging times but that is a significant contributor to exposures with a short averaging time. It could also exclude an exposure that is a minor component of most people's exposures but is a large fraction of a few people's exposures (i.e., high consumers). Proper attention to averaging time and the distribution of individual exposures (as advocated above) should make the second criterion moot.

The third bullet (on page 5) presents as a criterion for exclusion that a pesticide has low toxicity via the route in question. This criterion should also be rendered moot if toxicity-weighted exposures are used (as long as the weighing is route-specific). In fact, toxicity weighing is the only way to make such distinctions in a quantitatively sensible way, guaranteeing that excluded pathways make smaller risk contributions than included ones.

The fourth proposed criterion is that pesticide-pathway combinations may be excluded if the extent of allowable exposure is already limited by other kinds of toxic effects than those under consideration for the current CMG. Once again, the question about whether the exposure is sufficiently low to omit is a quantitative question that should be answerable with the first criterion, when properly formulated. The existence of the other mode of toxicity (and the use restriction it leads to) is merely a reason why the exposure can be supposed to be low.

A final point is that the question of exclusion is framed as though the various sources of exposure were fixed. In fact, in response to exclusions and the restrictions that may be put on a class of pesticides through risk analysis under the cumulative risk procedures, it may be that use patterns shift and market shares change such that exposures that may have been excludable initially may become relatively greater contributors to total cumulative exposure.

The issues of establishing viable and defensible criteria can also be approached from a different argument. As noted, an exclusion criterion based on a 1% rule does not, on its face, appear reasonable. The arbitrariness of such a criterion seems especially perverse in assessments whose purpose is to estimate cumulative exposures. Such a rule would allow an assessment to omit many small contributions even though they might, collectively, constitute the bulk of the exposure overall. In any case, it is hard to imagine how the rule will genuinely simplify or reduce the burden of the modeling and assessment process. It is difficult to judge the relative contributions until an analysis has been done anyway. In such a situation, it would be disingenuous to omit including a contribution merely because it is small. If the Agency adopts this or a comparable exclusionary criterion, it would be important to regularly revisit each exclusion whenever the assessment is updated or reconsidered. Whether a particular contribution appears to be below 1% of the total may well change as regulatory actions take effect and as new data become available.

Scientific judgment must be exercised when one selects which routes, pathways, and chemicals to include in an assessment model, and EPA cannot avoid responsibility for the modeling choices it makes with an arbitrary rule like the 1% exclusion criterion. When an effect is known to be zero, or is known to be essentially zero, it doesn't seriously complicate the assessment to include the value. Doing so helps to show that the assessment has been comprehensive and inclusive.

On the other hand, if, as the EPA staff suggests, the criterion is used to "defer" rather than " exclude" considerations during an ongoing and iterative modeling process, the application of the criterion might be entirely reasonable, especially if it improves the timeliness of assessments. The key in this case, however, will be to institute regular reviews. As yet, no specific schedule for review has been identified by the Agency, and this is a serious deficiency of the process articulated for cumulative assessments. Without a prescribed plan for revisiting the exclusions there is little justification for allowing them.

The Panel sympathizes with the desire to simplify the burden of modeling and assessment. Toward this end, it might be helpful to use a priori expectations about the respective sizes of different contributions to allocate modeling resources. The relative magnitudes of the contributions expected from different pathways, routes or chemicals could be used to apportion empirical effort afforded to each. For instance, if prior judgement about a particular pathway is that it contributes very little to the overall cumulative exposure, it would be reasonable to expend little time or empirical effort to get the best possible estimates for the parameters that characterize this pathway. Perhaps even a *relatively* broad interval estimated from the literature would suffice to represent the location and uncertainty about such a parameter. If the value is known to be negligible, then even overestimating the uncertainty would have little consequence for the quantitative results. As the expected magnitude of the contribution through a pathway increases, it would be reasonable to expend more effort to achieve a good estimate of the relevant parameters. It might be decided, for instance, to employ a panel of experts and a formal

elicitation procedure to estimate probability distributions to describe the locations and uncertainties about the parameters for pathways expected to have large overall contribution to the cumulative exposure. This kind of structured estimation procedure could be useful in lightening the burden of modeling cumulative exposures.

Comments were also made regarding the basic methodology used to summarize relative potency among the different chemicals in the CMG—a simple extension of standard NOAEL/LOAEL analysis. This way of assessing chemical potency is a basic mistake. NOAEL's are notoriously poor statistical properties as measures of the relative potency of different chemicals in causing specific effects in part because they are so dependent on experimental design in the form of the spacing of the doses chosen by the original investigators. Moreover, part of the point of assessing combined toxicity via a common mechanism is that one has the opportunity to (1) develop a full dose response relationship for one or more intermediates along the causal pathway to effect (in this case the intermediates would be inhibition of specific cholinesterases at various anatomical sites), (2) add up the expected effects of different chemicals as a function of dose in terms of the expected changes in each intermediate, and (3) interpret the likelihood of toxic effects as a function of dose in terms of the relationships of the intermediate parameters to the end responses of concern. Two possible approaches were suggested by a Panel Member regarding the case of cholinesterase inhibition. The first is a straightforward Michaelis-Menten enzyme inhibition model. The alternative is a slightly more complicated compartmental model to understand the dose response relationships for inhibition of each form of cholinesterase in each gender/species by each agent separately and then add up the expected intermediate responses as a function of dose for each modeled system.

In addition to better defining the criteria of "exclusion," a comment was made regarding the overall approach to "inclusion," using the case study in dietary exposure as an example. One of the underlying observations that sparked the concerns for cumulative risk from chemicals with common mechanism of toxicity was the realization that more than one pesticide are present in a single commodity, as was illustrated in the PDP data presented in the case study. However, as far as dietary exposure is concern, cumulative risk does not occur just through the co-occurrence of multiple pesticides in a single commodity, but also on the number of pesticides from the CMG that are present in a person's entire diet, which consists of many commodities and foods. Therefore, a better description of inclusion will be helpful for the dietary exposure pathway. It would consist of criteria for commodities and food ingredients based on the variety of foods people eat (i.e., based on the exposure patterns of individuals), rather than the traditional approach for a single pesticide assessment which focuses on pesticide-commodity pairs.

Issue #3: National and Regional Exposures

The potential for people to encounter overlapping exposures to different pesticides will be influenced by many factors. One important consideration is the geographic effects and seasonal uses of pesticides. Thus, a framework is proposed for assessing different pathways of exposure that are essentially driven by these considerations. OPP believes that the dietary (food) pathway should be approached on both a national and regional scale to

account for both national and regional distribution of treated commodities. However, the Office believes that residential and dietary (drinking water) pathways are more appropriately dealt with on a regional or multi-state basis, since there is no single, national source of drinking water; and residential exposures may be driven by regional use patterns.

Question 3: Please comment on whether the concept of developing a series of cumulative assessments on a geographic scale for different pathways is reasonable.

To the extent possible, the assessment of residential, nonoccupational, and institutional use patterns should characterize seasonal and geographical variations. Often the more significant pathway(s) of exposure to pesticides will be reflected by regional use characteristics. This is particularly true for residential and drinking water pathways of exposure. However, pesticides used on commercial crops intended for food consumption have a distribution that is often greater than regional, i.e., from point of pesticide use to food consumption.

This draft guidance needs to be defined and applied very carefully. In order for cumulative risk assessments to be accurate, the use patterns and practices on a scale sufficient to capture the variability in pesticide use need to be known accurately. How are the boundaries of the exposed population defined in such cases for calculating risk, i.e., the number of persons exposed for a given scenario? Are boundaries the same for each pesticide in the CMG for the pathways that are included in the exposure assessment? In other words, the "regions" applicable to each pesticide may be different even though they have a common mechanism of action. This issue potentially introduces a bias or an inaccuracy into the assessment as compared to many of the other issues which impact imprecision. Cumulative exposures for a case of herbicides will elicit several additional issues of defining the regional/local occurrence. This would broaden the perspective in developing criteria/guidelines for dealing with such scenarios . For example, ground water contamination (rural exposure) vs. surface (urban exposure), and residential (outdoor) exposures for children.

Given the caveats in the Agency background document about examining special populations and consumption patterns, it seems reasonable to perform national scale assessments for dietary exposures. Regional scale assessments for dietary, residential, and track-in exposures from agricultural sources are worthwhile but are more difficult due to the multiple scales at which data are collected. As has been stated, the sampling frames, i.e. the spatial boundaries under which data are collected, need to be carefully examined before they are combined. Common frames used, e.g., include census or political jurisdictions, may be too large for many types of special population assessments: statewide, or even county level frames may result in unintended consequences in model outputs for farm worker children if the counties originally sampled include a large urban area as well as more rural sections. These assessments need well defined and appropriates frames, in order to avoid the problem of combining multiple data sets collected at widely varying scales of aggregation.

The hypothetical scenarios provided for examples A-C (pages 13-14) are helpful in

describing possible scenarios that could occur. The case study is limited to food as the predominant pathway, and thus it is not clear what the results would be for the scenarios given in examples A-C. A case study should actually exercise the draft guidance by selecting a set of CMG pesticides and performing an exposure assessment with available extant data. When partitioning databases into smaller components, e.g., say subpopulations, the reliability of the data deteriorates because of the reduction in sample size. A case in point is food consumption in children. In the assessments, a display of the confidence intervals is very helpful to convey the strengths and weaknesses of the variety of scenarios that were assumed.

Another aspect of regional considerations for residential exposures is that geography is only one factor and probably not, in many cases, the most important way of subdividing the problem from the standpoint of modeling exposures. For example exposure to pet-related pesticides is probably best assessed by dividing the population up among those who do and do not have different kinds of pets. Additionally, sampling population weights should be used in the analyses of the dietary data, if this is not already the practice in Agency assessments.

It is important that spatial variability be considered carefully for all pathways. Spatial variation in pesticide use exists for all potential pathways: food ingestion, water ingestion, and residential and other indoor uses, and outdoor uses (such as the malathion spraying for West-Nile like virus in the New York City vicinity during the fall of 1999). The methods used should capture the full variability of cumulative exposures, identify the relative contribution of various pathways, and the relative importance of sources within pathways. This will facilitate the strategic targeting of regulatory practices toward the greatest sources of exposure and risk, within pathways.

Issue: Case Study

Cumulative risk assessment is at an early stage of development. Furthermore, there is very limited experience in conducting such assessments. Thus, the development of case studies using actual data are critical to refining useful and practical guidance, and to identifying future research and testing needs. OPP is taking a step wise approach to the development of such case studies by starting with simple examples and moving toward more complex situations.

Attached is a case study that uses actual dietary (food) residue data on three pesticides and evaluates only a single pathway/route/duration of exposure. Certain assumptions were made in the case study. In single chemical exposure assessments, for example, nondetects are assumed to be one-half the level of detection and composite samples are decomposited. In this case study, for illustrative purposes, nondetects were assumed to be zero, the samples were not decomposited, and surrogate data were not used.

Question 4: Given that an important goal of the cumulative assessment is to reliably determine sources of concern from a multi chemical exposure, please comment on to

what extent is it appropriate to apply standard practices and assumptions used in single chemical assessments.

Common Toxicology Group: Concerns were raised regarding the selection of pesticides in a CAG. Most insecticides are directed at targets in the nervous system which, as the command organ of the body, produces rapid insect kill. There are some different receptors and receptor subtypes between insects and humans, as well as differences in sensitivities of the same molecular target in insects and humans and in rates of degradation and elimination of the toxicants. These differences are utilized by the insecticide industry to develop insect-selective chemicals that are safer to humans.

The terms "common mechanism of action" and "common mechanism of toxicity" are used interchangeably without any distinction between them. There is a concern if only the common molecular target of a group of pesticides is considered, without attention to common toxicity symptoms. A common mechanism of action group includes chemicals that share molecular target(s). On the other hand, a common toxicity applies when similar toxicity symptoms are produced by attack on the same or different molecular targets. An example is the excessive stimulation of nicotinic receptors directly by action of neonicotinoids as well as indirectly by inhibition of acetylcholinesterase with organophosphate or carbamate anticholinesterases. Both kinds of insecticides activate nicotinic receptors in skeletal muscle. Its excessive stimulation by acetylcholine, that results from an anticholinesterase organophosphate, causes rapid desensitization of the nicotine receptor, producing muscle fibrillation and fasciculation that leads to severe weaknesses in breathing muscles (diaphragm and intercostal). Thus, these two groups of insecticides produce overlapping toxicities, since the anticholinesterases also affect the muscarinic receptor with excessive activation by the accumulating acetylcholine while neonicotinoids do not affect this receptor. This receptor desensitizes slowly, and toxicity symptoms are a result of activation of muscarinic receptors. These symptoms include excessive glandular secretions and miosis (due to activation of the excitatory muscarinic receptor subtypes M_1 and M_3) and lowered heart rate (caused by activation of the M_2 inhibitory muscarinic receptor in cardiac muscle). Therefore, insecticides may have different molecular targets, but share totally or in part in causing common toxicities. Accordingly, the common mechanism of toxicity of the anticholinesterases and neonicotinoids suggests that they should be grouped together, based on their common toxic action affecting only the nicotinic receptors.

Very few anticholinesterases also may have direct effect on muscarinic receptors. An example is parathion, which was shown to act directly on muscarinic receptors, having opposing effects on the two targets: high affinity activation of an inhibitory M_2 muscarinic receptor (Jett et al., 1991) and inhibition of acetylcholinesterase (which leads to activation of both nicotinic and muscarinic receptors). In summary, an insecticide may have more than one molecular target, thereby producing additive or reduced toxicity symptoms. Overlap may exist for exposures, as well as for the toxicities of neuroactive chemicals.

There are also totally indirect interactions between insecticides. An example is pyrethroids

(Type I and Type II) that keep axonal sodium channels open longer (Narahashi, 1985), but only Type II pyrethroids also inhibit the inhibitory gamma-aminobutyric acid receptor (Lawrence and Casida, 1983). Each of these actions produces excitation in the nervous system. Another example of excitation is that produced by lindane and the cyclodiene endosulfan, because both inhibit the inhibitory gamma-aminobutyric acid receptor (Gant *et al.*, 1987). Thus, Type II pyrethroids, lindane and endosulfan also should be considered for grouping together. This is yet another example for a common toxicity group.

It is important to realize that there is cross-communication between brain proteins. Thus, in order to counteract the convulsions produced by anticholinesterases, diazepam (which inhibits Na^+ channels and potentiates inhibitory gamma-aminobutyric acid receptors) is an effective antidote.

Methods used in the Case Study: Several of the simplifications made in the case study analysis would be unwise if carried forward into an analysis that was intended to be used for real identification of chemicals and pathways of concern and the needs for additional regulatory exposure reduction measures. There should be a basic set of distributional analysis of exposures that represents the best assessments that can be done for both exposure variability by multiple routes and the uncertainties in the variable exposure distributions for each agent and route. Non-detects in the dietary exposure route should not all be represented as zero's, but there should be some (usually lognormal) distribution inferred for the fraction of each crop that is treated, although mixtures of lognormals should also be considered with appropriate statistical tools. Additionally, pathways other than food can be included in the analysis without undue extra effort using the technique outlined in the response to Question 1. Decompositing procedures as developed and discussed at the previous SAP meeting should be used to reconstruct distributions of single serving residues.

One panel member noted that the Agency was provided a Monte Carlo approach to cumulative exposure and risk assessment by the 1993 NAS Committee that produced the report, *Pesticides in the Diets of Infants and Children*. This approach remains valid, is easily replicated, and should be extended to additional pathways and chemicals when a common mechanism of action is to be presumed. Choice of treatment of non-detections had little effect on cumulative exposure in the NAS study. Assumptions made concerning the transfer of residue from raw commodities to juices made a significant difference in exposure estimates. (NAS 1993: 297-307). This type of Monte Carlo assessment should be quickly adopted for organophosphates and combined with sensitivity tests to determine the relative contributions of individual chemicals and pathways to cumulative exposure and risk. All of the problems concerning variability in data quality will be compounded in multi-chemical assessments; however, this is not a logic to avoid the assessment. Instead it is reason to clearly state what will be assumed when data are deemed to be of insufficient quality for use in quantitative assessments.

Finally, the NOAEL/LOAEL-based modeling of relative potency should be

replaced with an assessment of the potency of the variable of interest—inhibition of cholinesterases—as quantitative parameters in their own right. Low dose potency (potencies) should be calculated for inhibition of these enzymes. The ultimate human analysis should be conducted in terms of how many people are likely to suffer what percentage change in their cholinesterase levels after appropriate animal-to-human dosimetric adjustments and considerations involved in modeling the dynamics of cholinesterase inhibition and regeneration in humans.

FQPA Safety Factor: The case study does not illustrate adequately the issues regarding appropriate use of the FQPA safety factor. There is some discussion of data relevant for determining whether increased sensitivity from pre- or post-natal exposure exists for the hypothetical chemicals (sections 2.24, 2.3.4, and 2.4.4. of the draft guidance), and (conveniently) none showed evidence of special risk for children. Presumably, it was decided that a FQPA safety factor was not needed for this cumulative assessment, although this is not explicitly stated in the case study. As the case studies used by the Agency to illustrate cumulative risk assessment guidance evolve and come to more closely resemble "real world" examples, it will be important to more clearly demonstrate how the FQPA safety factor might be applied on a group basis. In Section 6 of the draft guidance (page 29), the application of a FQPA safety factor to the CAG is discussed. It states here that the decision to apply a FQPA safety factor depends on the common mechanism of the group, and that the FQPA is therefore not chemical-specific. This oversimplifies the situation. Special susceptibility of children is a function not only of the mechanism of toxicity but also of the biological disposition of the chemical (distribution, metabolism, elimination, etc.), particularly as it pertains to prenatal and perinatal exposure. Biological disposition can be quite chemical-specific, conceivably even for chemicals that share a common mechanism of toxicity (or, more accurately termed as it pertains to chemicals within a CMG, mode of toxicity). As examples, transplacental distribution of chemicals and their appearance in breast milk can vary substantially and are important considerations in potential prenatal and perinatal susceptibility to toxicity. It is unclear from the current guidance how differences in these factors among members of a CAG would be addressed in determining whether a FQPA safety factor should be applied. In future versions of the guidance, greater attention to the development and application of the FQPA safety factor is warranted.

Validation:

At some point in the process, the Agency is encouraged to validate its estimates of the cumulative exposures methodology. This could best be done by conducting the cumulative exposure estimates and then following this with actual human monitoring data. The ideal chemical to conduct this on would be one that has a readily identifiable biomarker, e.g., the parent chemical itself or metabolite. However, the validating chemical would not necessarily have to be a pesticide, although it would be preferable if such a pesticide could be identified. It is only through a study such as this that the Agency and the public at large can have confidence in these estimates. An unreliable estimate is worse than no estimate at all because of its long-lasting effect on the credibility of the process.

REFERENCES

-Gant , D., M. Eldefrawi and A. Eldefrawi (1987). Cyclodiene insecticides inhibit GABA receptor-regulated chloride transport. *Tox. Appl. Pharmacol*., 88:313-321.

-Jett, D., E. Abdallah, E.El-Fakahany, M. Eldefrawi, and A. Eldefrawi (1991). High affinity activation by paraoxon of muscarinic receptor subtype in rat brain striatum. *Pestic. Biochem. Physiol*., 39:149-157.

-Lawrence, L and J. Casida (1983). Stereospecific action of pyrethroid insecticides on the gamma-aminobutyric acid receptor-ionophore complex. *Science,* 221:1399-1401.

-Narahashi, T. (1985). Nerve membrane ionic channels as the primary targets for pyrethroids. *Neurotoxicol.,* 6:3-22.

-National Academy Press (1993). Pesticides in the diets of infants and children. Committee on Pesticides in the Diets of Infants and Children, National Research Council, 297-307.

APPENDIX

The following recommendation was provided by one Panel member in response to the Agency's issue of combining higher quality monitoring data with more limited screening level data.

The stated concern is that the value and benefit of high quality monitoring data will be lost if combined with extrapolated exposure values from poor quality data. One panel member commented that, since of the three general pathways (i.e., dietary-food, drinking water, and residential), the quality of data is generally better for dietary-food, this guideline leads to the apparent position that most exposure assessments will be driven by food. This was illustrated by considering the following scenario regarding data quality:

Pathway	Pesticide A	Pesticide B	Pesticide C
Dietary-Food	+/G	+/G	-
Drinking Water	-	+/P	+/P
Residential	-	-	+/P

In this case, the quality of data for the dietary pathway for Pesticides A and B is considered good ("G"). Drinking water and residential pathways are not considered important contribution to exposure for Pesticide A (as represented by "-"). Pesticide B appears in drinking water (represented by "+"), but the quality of data is poor ("P") and thus appears to be less important in the residential pathway. Exposure to Pesticide C occurs in both drinking water and residential pathways but the quality of data is poor in both cases. Under this set of circumstances, and following the proposed guidance, the only pathway that cumulative exposure would be combined is that from dietary. It would be helpful to also show example case(s) where the data quality for a pathway is poor but drives the overall assessment.