A Set of Scientific Issues Being Considered by the Agency in Connection with the Revisions of Toxicity Guidelines (includes acutes, subchronics, mutagenicity, neurotoxicity and chronic/oncogenicity) under Health Effects Test Guidelines OPPTS 870.1000 Acute Toxicity Testing

A Set of Scientific Issues Being Considered by the Agency as to Whether an Additional Uncertainty Factor is Necessary and Appropriate to Assess Pre- and Post-Natal Development and Reproductive Effects in Infants and Children Exposed to Pesticides through Chronic Dietary Exposure (10x)

A Set of Scientific Issues Being Considered by the Agency as to the Effects of Acute Inhalation Toxicity with Histopathology (870.1350)

A Set of Scientific Issues Being Considered by the Agency in Connection with the Metabolism Guidelines under the Health Effects Guidelines OPPTS 870.7485 Metabolism and Kinetics

A Set of Scientific Issues Being Considered by the Agency in Connection with the Immunotoxicity Guidelines under the Health Effects Test Guidelines OPPTS 870.1000

A Set of Scientific Issues Being Considered by the Agency in Connection with the Domestic Animal Safety Guidelines under the Health Effects Test Guidelines OPPTS 870.7200 Domestic Animal Safety

A Set of Scientific Issues Being Considered by the Agency in Connection with the Comparison of the Effects of Chemicals with Combined Perinatal and Adult Exposure vs. Adult Only Exposure in Carcinogenisis Bioassays

A Set of Scientific Issues Being Considered by the Agency in Connection with the Health Effects Test Guidelines OPPTS 870.3700 Prenatal Development Toxicity Study and the Health Effects Test Guidelines OPPTS 870.3800 Reproduction and Fertility Effects

A Set of Scientific Issues Being Considered by the Agency to Discuss and Evaluate the Weight-of-Evidence for Vinclozolin with Particular Reference to its Potential for Developmental and Reproductive Toxicity

A Set of Scientific Issues Being Considered by the Agency to Discuss and Evaluate the Weight-of-Evidence for Vinclozolin with Particular Reference to its Carcinogenic Potential

A Set of Scientific Issues Being Considered by the Agency to Discuss and Evaluate the Weight-of-Evidence for Alachlor with Particular Reference to its Carcinogenic Potential

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

and

SCIENCE ADVISORY BOARD

JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency in Connection with the Revisions of Toxicity Guidelines (includes acutes, subchronics, mutagenicity, neurotoxicity and chronic/oncogenicity) under Health Effects Test Guidelines OPPTS 870.1000 Acute Toxicity Testing.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues regarding Revisions of Toxicity Guidelines. The review was conducted in an open meeting held in Arlington, Virginia, on October 29, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Marion W. Anders (University of Rochester Medical Center); Dr. Charles C. Capen (Ohio State University); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); Dr. Bernard Weiss (University of Rochester).

Public Notice of the meeting was published in the Federal Register on 28 August, 1996.

Oral statements were received from:
Dr. Abe Tobia, American Crop Protection Association
Dr. Donald R. Saunders, Ciba-Geigy Corporation
Ms. Annette M. Kirk, Ceregen

Written statements were provided by:
Gary Wnorowski - Chemical Producers and Distributors Association
Gerald G. Long - Society of Toxicologic Pathologists
American Crop Protection Association

SAP'S GENERAL COMMENTS ON THE GUIDELINES

The SAP commends the Agency for its efforts to develop revised guidelines and to harmonize the present inter, intra, and international guidelines. Although harmonization of guidelines is a worthy effort, the Agency needs to ensure that the harmonization process does not prevent the use of good experimental design in developing and promulgating revised guidelines. In addition, the guidelines must be flexible enough to accommodate new scientific advances as they become available. Whenever possible, the Agency should work with industry and academia to evaluate and publish (within the bounds of confidentiality) the results of various types of studies to establish a definitive database that can be used to determine the likely usefulness of new guidelines. It is recommended that the Agency refrain from the use of anecdotal observations in developing revised guidelines.

As an example of needed flexibility in guidelines, although the Agency prefers dosing with constant concentrations rather than with constant volumes, the Agency should state its preference for dosing with constant concentrations and the basis for its preference, but should be flexible enough to accept dosing with constant volumes when the conduct of the study clearly demands this approach.

The number of animals used per dose or study group differs among the guidelines being harmonized. The Agency should, however, strive to use the fewest animals possible to achieve maximum scientifically valid results.

The use of control groups that provide the best experimental design should be required. This may require the use of both naive and vehicle control groups in some studies. The need for a naive or vehicle control group should be waived only if existing data clearly support the waiver.

In the discussion about the chronic toxicity guidelines (OPPTS 870.4100), the requirement for clinical pathology data, including organ weights, hematology, and clinical chemistry, beyond the 12-month observation period was considered. The SAP asserts that such data are of questionable value, particularly as they relate to noncancer endpoints. (The SAP is aware that a 12-month observation period is indicated in the guidelines and endorses that observation period for noncancer endpoints.)

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

_____
LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

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JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency as to Whether an Additional Uncertainty Factor is Necessary and Appropriate to Assess Pre- and Post-Natal Development and Reproductive Effects in Infants and Children Exposed to Pesticides through Chronic Dietary Exposure (10X).

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues regarding the 10X Uncertainty Factor for Developmental/Reproductive Effects. The review was conducted in an open meeting held in Arlington, Virginia, on October 29, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Marion W. Anders (University of Rochester Medical Center); Dr. Charles C. Capen (Ohio State University); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); (Dr. Bernard Weiss (University of Rochester).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

Written statements were provided by:
American Crop Protection Association
Oral comments provided by:
Dr. Abe Tobia, American Crop Protection Association

THE USE OF AN EXTRA UNCERTAINTY FACTOR TO ASSESS

PRE- AND POST-NATAL DEVELOPMENTAL AND REPRODUCTIVE EFFECTS IN INFANTS AND CHILDREN EXPOSED TO PESTICIDES THRU CHRONIC DIETARY EXPOSURE

EPA's Questions for the SAP's Consideration with Their Recommendations

QUESTION 1: ADEQUACY OF THE WEIGHT-OF-THE-EVIDENCE APPROACH

Currently, the OPP uses a weight of the evidence approach of applying additional uncertainty factors in setting the reference dose when a potential unique risk for neonates and infants is identified from the standard reproduction, developmental and developmental neurotoxicity tests in conjunction with subchronic and chronic data. Is this approach adequate for potential effects on neonates, infants, and children?

SAP RECOMMENDATION

The NAS report on children and pesticides recommended an additional 10-fold uncertainty factor. The Agency position that a 10-fold uncertainty factor (UF) should not be applied in every case is reasonable. This is a growing area of toxicology in which there are numerous data gaps and uncertainties. Therefore, the circumstances under which greater or lower UFs would be made are not clear and cogent. The Agency notes that additional UFs may be applied in the absence of complete information. How is an incomplete data base defined? Is it restricted to the guideline data only? Without additional information or the use of additional testing, it would be difficult to assign a narrowly defined UF. As long as the shallowness of the information underlying the guidelines persists, it may be necessary to invoke default values and higher UFs.

Each pesticide needs to be evaluated on a case-by-case basis focusing on the available database using a weight of evidence approach for each uncertainty factor decison.

QUESTION 2: ADDITIONAL FACTORS

If not, what additional factors need to be addressed?

SAP RECOMMENDATION

The Agency should make use of emerging human information on the risks of low level exposures to such agents as lead to evaluate whether current testing guidelines are sufficiently sensitive to detect such risks in advance of human exposure.

Further, in calculating risks one should take account of differences between adults and children in exposure to different amounts of a single chemical and to multiple agents. The EPA report, Environmental Health Threats to Children, notes that the Agency plans to "move beyond the chemical-by-chemical approaches of the past, so that we can address cumulative and simultaneous exposures."

QUESTION 3: ADEQUACY OF THE PROPOSED GUIDELINES

Are the proposed modifications to the prenatal developmental and reproduction testing guidelines sufficient to allow OPP to adequately assess pre- and postnatal toxicity? If not, what additional measures could be added to these newly-revised guidelines in the short-term? What modifications could/should be made over the long-term?

SAP RECOMMENDATION

Neurotoxicity is not well integrated with the developmental guidelines. For example, schedule-controlled operant behavior is a separate guideline. Is some kind of integration foreseen in the future? Has the Agency examined the pesticide literature in sufficient detail to ascertain whether the proposed guidelines would yield data in conformity with what experimenters have determined to be sensitive endpoints? For example, would a guideline assessment, of chlordane, say, produce results consistent with the paper by Cassidy et al, Toxicol. App. Pharmacol. 126:326-337,(1994)? Also, the guidelines seem not to ask for distributions; instead, they focus on measures of central tendency. Is this an appropriate way to examine data for human risk assessment when adverse effects in human populations may be expressed primarily by shifts in the shape of the distribution or when minor central tendency shifts produce major changes at the extremes of the distribution? Such perspectives on risk have been proposed as crucial for weighing the neurotoxic hazards of lead, and immunotoxicologists have proposed that decreases in immune function be viewed in the same way.

The Agency may want to conduct a review of the available

literature on the value of in-utero exposure for detecting neurotoxicity similar to the review conducted for carcinogenicity.

FOR THE CHAIRMAN:

Cerified as an accurate report of Findings:

_____

LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

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JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency as to the Effects of Acute Inhalation Toxicity with Histopathology (870.1350).

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of the effects of Acute Inhalation Toxicity with Histopathology. The review was conducted in an open meeting held in Arlington, Virginia, on October 29, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Marion W. Anders (University of Rochester Medical Center); Dr. Charles C. Capen (Ohio State University); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); (Dr. Bernard Weiss (University of Rochester).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

Oral statements were received from:
Dr. Elliott B. Gordon, Makhteshim-Agan of North America

ACUTE INHALATION GUIDELINE (870.1350)

EPA's Questions for the SAP's Consideration with Their Recommendations

The SAP Panel was in general agreement with the proposed guidelines to evaluate acute toxic effects of substances for which the lung is the portal of entry, by inhalation exposure route.

New advances in the utility of bronchioalveolar lavage techniques can be applied to evaluating toxic effects from inhalation exposure. The question of whether inhalation exposure or intratracheal instillation is used is largely addressed by the nature of the substance to be tested. In general, testing by inhalation is preferable to intratacheal instillation. This is particularly true for particles and fibers. In addition, the SAP expresses some reservation as to whether testing fibrous and non-fibrous particulates via inhalation exposure for a short duration would provide meaningful information for risk assessment. In most cases a minimum of 28 days and preferably 90 days will be required for particulates.

In the definition section of the inhalation toxicity guidelines, it was recommended that the 'target organ' be defined. While the primary target organ is the lung, it is recognized that effects on other tissues and organs are also possible.

Provided below are the Panel's recommendations regarding the two questions that the Agency posed.

QUESTION 1: EXPOSURE TIME PERIOD

Although EPA agrees with commenters that a one hour exposure is technically difficult and is considering dropping this requirement from the final version of the acute inhalation guideline (870.1350), the Agency is interested in the C X T relationship so it can extrapolate from one acute exposure time to another. Thus, EPA is still considering the 4 hour exposure and one longer exposure period so that the effects of time exposure can be experimentally determined. Is this a valid approach? If so, what other exposure time should be required?

SAP RECOMMENDATION

The Panel supports the Agency's proposal to eliminate the 1-hour exposure and to retain the 4-hour exposure test and also to include another exposure time point such as 8-hour exposure. Twenty-four hour exposure is not likely to yield much useful information because of complicating factors such as biological rhythms and will not be appropriate for relatively insoluable or chemically inactive paticulates.

QUESTION 2: CONSERVING TISSUE SAMPLES

In an effort to balance expense and a concern for potential systemic effect resulting from acute exposure, EPA is requiring that tissue samples from the acute study be reserved and examined retrospectively if positive results are found in subchronic studies. Although this is not a conventional approach, EPA believes that a better understanding of the early events of toxic insult may be obtained by examining tissues from the acute study corresponding to those in which lesions were identified in the subchronic. In your opinion, is this approach reasonable and scientifically sound?

SAP RECOMMENDATION

The proposed revision balancing the expense and a concern for potential systemic effects resulting from acute inhalation exposure is scientifically sound.

When positive results are found from the subchronic studies, the tissues from the acute study may be required to be examined by histopathology, retrospectively. This should not impose any difficulties since GLP requires that all tissues from the acute study be preserved for retrospective examination. This will facilitate identification of target organs/tissues as needed without undue burden.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

_____

LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

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JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency in Connection with the Metabolism Guidelines under the Health Effects Test Guidelines OPPTS 870.7485 Metabolism and Kinetics.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues regarding the Metabolism Guidelines. The review was conducted in an open meeting held in Arlington, Virginia, on October 29, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Marion W. Anders (University of Rochester Medical Center); Dr. Charles C. Capen (Ohio State University); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); Dr. Bernard Weiss (University of Rochester).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

METABOLISM GUIDELINE (OPPTS 870.7485):

EPA's Question for the SAP's Consideration with Their Recommendations

QUESTION: Does the SAP agree that the revised Metabolism Guideline, employing a tiered system, provides a reasonable and adequate approach to metabolism testing for OPPTS?

SAP RECOMMENDATION

The revised metabolism guidelines are designed to provide information on the absorption, distribution, biotransformation, and excretion of test substances and to aid in the understanding of the mechanism of toxicity. The SAP regards these guidelines as timely and necessary. Moreover, the guidelines are practical and flexible enough to suffice for a range of chemical compounds.

The agency should consider these specific points about the guidelines:

1. Three young adult male animals, rather than four, should be sufficient for Tier 1 testing.

2. The usefulness of tissue distribution studies seven days after treatment or after 90% of the administered dose has been recovered is questionable. The SAP is concerned that the concentration of compound in tissues may be too low to be meaningful. In addition, the guideline does not specify whether the concentration of free or bound compound or parent compound or metabolite should be quantified.

3. The outcomes of Tier 1 tests that trigger Tier 2 tests are not clear. In addition, the objectives of all of the Tier 2 tests are not clear.

4. The objective of the Tier 2 tests on the tissue-distribution time course or plasma kinetics is not clear. Furthermore, the guideline does not specify whether the concentration of free or bound compound or parent compound or metabolite should be quantified. The guideline should state explicitly that measurements of uncharacterized radioactivity will not satisfy the guidelines. Although not stated in the guidelines, it is presumed that these guidelines would be most useful for understanding noncancer endpoints.

5. The SAP endorses the use of physiologically based pharmacokinetic modeling studies. To encourage the use of PBPK models, it should be possible to submit results of PBPK studies of the parent compound in lieu of some of the other metabolism studies.

In summary, the SAP agrees that the revised metabolism guideline, employing a tiered system, provides a reasonable and adequate approach to metabolism testing for OPPTS.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

_____
LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

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JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency in Connection with the Immunotoxicity Guidelines under the Health Effects Test Guidelines OPPTS 870.1000

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues regarding the Immunotoxicity Guidelines. The review was conducted in an open meeting held in Arlington, Virginia, on October 29, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Marion W. Anders (University of Rochester Medical Center); Dr. Charles C. Capen (Ohio State University); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); (Dr. Bernard Weiss (University of Rochester).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

Written Comments were received from:
Health and Environmental Sciences Institute

Oral statements were received from:
Dr. Abraham Tobia, American Crop Protection Association
Dr. Scott Loveless, DuPont Haskell Laboratory

IMMUNOTOXICITY GUIDELINE (OPPTS 870.7800):

EPA's Issues for the SAP's Consideration with Their Recommendations

The purpose of this meeting was for the EPA SAP and the public to provide input into the draft guidelines for immunotoxicity testing (OPPTS 870.780). Following a presentation by the EPA, the floor was open for discussion of several issues related to the draft guidelines. These issues were:

1) Removal of the requirement for immune cell phenotyping and recommending one of the three proposed Options;
2) Clarification on the wording for the "optional immunotoxicity screen" para. in the various subchronic and chronic toxicity studies proposed by EPA;
3) Clarification of monoclonal antibodies for use in immune cell phenotyping;
4) Clarification for route of test article exposure;
5) Clarification of the numbers of animals/group;
6) Clarification of route of immunization; and
7) Clarification of frequency of inclusion of a positive immunosuppression control.

The SAP reiterated its belief that the immune system is a legitimate target organ for toxicity. Furthermore, it is felt that the methodology is sufficiently validated in both the rat and mouse systems for inclusion into routine toxicology hazard evaluation studies.

Provided below are the seven Issues that EPA presented to the SAP along with the SAP's recommendations. In addition to the seven issues, a number of other comments were made by SAP. These comments follow the issues.

ISSUE 1: REMOVAL OF THE REQUIREMENT FOR IMMUNE CELL PHENOTYPING

The draft immunotoxicity guideline requires an antibody assay (via Plaque Forming Cell (PFC) assay or ELISA) after 30 days of exposure, and an assessment of peripheral blood total T-cells, total B-cells, T-cell subsets CD4 and CD8, and NK cells (via flow cytometry) after 90 days of exposure (Section (d)(1) & (2)). Outside comments have indicated that phenotypic analysis may not provide sufficient information to merit inclusion as a requirement in the guideline, especially for the enumeration of NK cells.

In addition, the draft immunotoxicity guideline states that "rats and/or mice" (Section (d)(1)) are the species to be used in the immunotoxicity screen. EPA recommends that the guideline be amended to require that both mice and rats be tested. The rationale for this is that there are compounds which are immunotoxic in rats but not mice, and vice versa.

The draft immunotoxicity guideline also states in Section (d)(1) that the PFC/ELISA assay

be performed after "at least 30 days" of dosing. Several outside comments have indicated that performance of this test at 28 days of exposure is preferred; thus, the EPA recommends that the time frame be changed to allow the range of 28-30 days post-dosing.

EPA would like the FIFRA SAP to consider the following options, and make a recommendation for amending the guideline:

A. Eliminate the requirement for flow cytometric analysis of lymphocyte and NK cell phenotypes. Require only an antibody to SRBC assay, in rats and mice, after 28-30 days of dosing.

-- This option would yield good information about several functional aspects of the immune system, including antigen presentation by macrophages, T-helper cells, and B cells, but will not demonstrate a chemical's effect on NK cell activity.

B. Eliminate the requirement for flow cytometric analysis of lymphocyte and NK cell phenotypes. Require an antibody to SRBC assay, by either PFC or ELISA, and a functional assay for NK cells after 28-30 days of dosing; both tests would be performed in rats and mice.

-- This option would provide data which could be used to show the effects of a chemical on NK cell function. If an NK cell activity assay is incorporated as a requirement, then a protocol similar to that found in the biochemical immunotoxicity guideline (OPPTS 880.3550) with appropriate reference(s) would be added to the draft immunotoxicity guideline (OPPTS 870.7800).

C. Eliminate the requirement for flow cytometric analysis of lymphocyte and NK cell phenotypes. Require an antibody to SRBC assay, by either PFC or ELISA, and a splenic NK cell activity assay; both tests would be performed in mice and rats. If any adverse effects are observed, the registrants would have the option of performing phenotypic analysis of total T, total B, and T cell subsets by flow cytometry, in the affected species. This test could be done in a separate group of animals, at 28-30 days of dosing, or incorporated into a 90-day subchronic oral, dermal, or inhalation toxicity test.

-- Option C, which includes the option of phenotypic analysis by flow cytometry, might be useful in interpreting the type(s) of lymphocyte subpopulations affected, if effects are observed in the functional PFC test at 28-30 days post-dosing. If the species affected was the rat, this could be incorporated into a standard 90-day test, without requiring additional animals, since the rat is the usual rodent species tested in subchronic

studies. However, if an adverse effect was observed in the mouse and not in the rat, then a separate group of mice would have to be tested. In addition, EPA has concerns that phenotypic analysis at 90 days post-dosing might not reflect the cell population(s) affected in the SRBC test at 28-30 days post-dosing.

The purpose for eliminating the lymphocyte and NK cell phenotyping as a requirement is that flow cytometry is costly, requires rigorous quality assurance, and highly trained technical assistance. In addition, cell surface markers for NK cells have not been validated, nor have studies been done to show that NK numbers, as measured by flow cytometry, are good indicators of immunotoxicity.

EPA requests the Panel's recommendation of one of the options listed above, and their comments on the number of species and time frame for performing the anti-SRBC assay (PFC or ELISA). If Option C is preferred by the Panel, EPA further requests advice as to when the phenotypic analysis should be performed.

SAP RECOMMENDATION

With regard to Issue 1, the SAP felt that the requirement for immune cell phenotyping should be dropped as a requirement and made an option. As such, the SAP recommends that the EPA choose Option C with inclusion of thymus weights. The issue of thymus weights was brought up in public comment, as this additional parameter limits the use of the animals. Briefly, an experimental design scenario which would include measuring the IgM response to sRBC at 28-30 days followed by looking for a memory response, NK activity or phenotying of lymphocytes at a later time period (i.e., 90 days) would be excluded by weighing the thymus at 28-30 days. This point was well taken by the SAP and if the registrant proposes to perform this type of study, the thymus weight should be weighed at the 90 day period. Furthermore, the issue of timing of phenotypic analysis relative to the AFC test was a concern to the Agency if Option C was selected and effects were seen in mice. The SAB believes that most immunotoxic changes, if they occur, will be noted after 28-30 days. Therefore, the registrant should have the flexibility to perform phenotypic analyses at this time rather than at 90 days. If ADME data suggest a longer exposure time frame is needed, the 90-day dosing period may be necessary. With respect to species selection, the SAP felt that either rats or mice could be used if there were data demonstrating similar ADME for the test compound in question. If such data were lacking, both species should be used.

ISSUE 2: CLARIFICATION ON THE "OPTIONAL IMMUNOTOXICITY SCREEN" PARAGRAPH

Currently, several of the Health Effects Test Guidelines have an "Optional immunotoxicity screen" paragraph: the 21/28 day repeated dose dermal toxicity test (OPPTS 870.3200); the 90 day oral (OPPTS 870.3100), dermal (OPPTS 870.3250), and inhalation (OPPTS 870.3465) studies; the chronic toxicity study (OPPTS 870.4100); the carcinogenicity study (OPPTS 870.4200), and the combined chronic/carcinogenicity study (OPPTS 870.4300). The paragraph in each of these studies reads as follows:

> "Optional immunotoxicity screen. In partial fulfillment of requirements for an immunotoxicity screen, subpopulations of splenic or peripheral blood lymphocytes in the rodents should be enumerated and quantified. Total T-, Total B-, Total T-helper,T-suppressor/cytotoxic and Natural Killer (NK) cell populations should be determined on at least 10 rodents of each sex in each group at the end of [the study]"

- for the repeated dose dermal toxicity study, this is 21 or 28 days
- for the subchronic studies, this is 90 days
- for the chronic study, this is 12 months
- for the carcinogenicity and combined chronic/carcinogenicity studies, this is two years for rats and 18 months for mice

The time points listed above for the studies longer than 90 days are inconsistent with the draft immunotoxicity guideline, which reads as follows:

> (f)(5) Administration of the test substance ".... A dedicated group of animals is not required for flow cytometric analysis. Under ordinary circumstances, this test should be done after 90 days of administration; however, if phenotypic analysis is performed in conjunction with a repeated dose dermal toxicity study, a shorter administration period may be allowed."

If flow cytometry is eliminated altogether, the "Optional immunotoxicity screen" paragraph will drop out of all of the studies listed above. However, if the Panel recommends that phenotypic analysis of lymphocytes become optional as described in Issue 1. Option C above, and that it should be incorporated into the 90-day subchronic studies, the "Optional immunotoxicity screen" paragraph in each of the subchronic studies should be amended as follows:

> Optional immunotoxicity screen. "If adverse effects are observed in either the rat or the mouse in a 28-30 day immunotoxicity study (OPPTS 870.7800), then subpopulations of splenic or peripheral blood lymphocytes in the species affected should be enumerated and quantified. Total T-, Total B-, Total T-helper, and T-suppressor/cytotoxic cell populations should be determined on at least 10 rats of each sex in each group at the end of the study."

In addition, if phenotypic analysis of lymphocytes is optional as described in 1. c. above,

then EPA also recommends that Section (f)(5) in OPPTS 870.7800 should also be amended, as follows:

> (f)(5) Administration of the test substance".... A dedicated group of animals is not required for flow cytometric analysis. When performed, phenotypic analysis of lymphocytes may be done after 90 days***** of administration, in conjunction with an appropriate subchronic oral (OPPTS 870.3100, dermal (OPPTS 870.3250), or inhalation (OPPTS 870.3465) study."
>
> *** NOTE:** The time frame for performing this test is contingent upon the recommendations of the panel in Issue 1, above.

EPA requests the Panel's comments and recommendations on this issue.

SAP RECOMMENDATION

With regard to Issue 2, clarification on the "Optional Immunotoxicity Screen" paragraph, the SAP agrees that adoption of Option C would necessitate the wording changes recommended by the EPA.

ISSUE 3: CLARIFICATION OF PHENOTYPING REAGENTS

The immunotoxicity guideline states in Section (d)(2) that "expression of phenotypic markers for major lymphocyte populations (total T (CD3), total B (CD-45R)...and T subpopulations (CD4 and CD8) as assessed by flow cytometry, is used to determine the effects on either splenic or peripheral-blood lymphocyte populations." Outside comments have disagreed with the use of the CD3 and CD-45R for quantitating total T and B cells, respectively, in the rat. Recommendations for species specific markers (e.g., clone W3/25, which produces anti-CD4 to quantitate T helper cells in the rat) have been made. To allow for future changes in hybridoma technology and in cell-surface marker identification, EPA would prefer to amend Section (d)(2) as follows:

> (d) Principle of the test methods. (2) Expression of phenotypic markers for major lymphocyte populations (Total T and Total B) and T-cell subpopulations (T Helpers (CD-4), T cytoxic/suppressors(CD-8)) as assessed by flow cytometry, is used to determine the effects on either splenic or peripheral-blood lymphocyte populations. The appropriate monoclonal antibodies should be used, which are specific for the species being tested.

EPA requests the Panel's comments and recommendations on this proposed amendment.

SAP RECOMMENDATION

With regard to Issue 3, clarification of phenotyping reagents, the SAP agrees with the Agency's recommendation that appropriate species-specific monoclonal antibodies be used. This will allow registrants sufficient flexibility to allow for advances in flow cytometry and antibody marker technology.

ISSUE 4: RECOMMENDED EXPOSURE ROUTE

The draft immunotoxicity guideline, Section (f)(5), states that the route of exposure will be "usually by the oral route." EPA suggests that route of exposure determined by the likely route of exposure of the test compound to human population. For occupational and indoor exposures, dermal or inhalation exposure may be more relevant. If the most relevant exposure is inhalation, we suggest a reference be included to the Air Toxics test rule. If the most relevant exposure route is dermal, then a reference to the "Administration of the test substance paragraph (Section (e)(8)) of the 21/28 day repeated dose dermal toxicity study guideline should be included, as well.

EPA requests the Panel's comments and recommendations on this issue.

SAP RECOMMENDATION

With regard to Issue 4, recommended exposure route, the SAP agrees with the Agency's recommendation that the route of exposure to the test compound be that most likely encountered by the consumer.

ISSUE 5: NUMBER OF ANIMALS PER GROUP

The draft immunotoxicity guideline, Section (f)(1)(iv), states that the number of animals used for each dose and control group should be 10 for the anti-SRBC PFC assay or ELISA, and 6 for the phenotypic analysis. This is inconsistent with other repeated-dose studies, which require 10 animals per sex per dose and control group. However, this may be more animals than is required for the anti-SRBC PFC (or ELISA) and the phenotypic analysis assays.

EPA requests the Panel's comments and recommendations on this issue.

SAP RECOMMENDATION

With respect to Issue 5, number of animals per group, the SAP felt that a minimum of 8 animals per treatment group would likely yield sufficient statistical power to detect a 20% change based upon the interanimal variation usually encountered in these assays.

ISSUE 6: ROUTE OF ADMINISTRATION

The draft immunotoxicity guideline, Section (g)(1)(i)(A), states the sheep red blood cells should be via the intravenous route. EPA recommends amendment of the guideline to include "or intraperitoneally," since this is an acceptable method for immunization with SRBCs.

EPA requests the Panel's comments and recommendations on this issue.

SAP RECOMMENDATION

Issue 6 focused on route of immunization. Since comparable immune responses were achievable following either intravenous or intraperitoneal injection of antigen, the Committee felt that either route was acceptable. If the intraperitoneal route is used for injection of antigen, the study director should be aware that a low percentage of animals may not respond because the antigen was inappropriately injected into the intestinal tract. The Agency should consider a minimally acceptable AFC response for the $B_6C_3F_1$ hybrid mouse. A response less than 800-1000 PFC/10^6 spleen cells could be the cut-off response in control mice.

ISSUE 7: USE OF POSITIVE CONTROL

The draft immunotoxicity guideline, Section (f)(3)(iii), addresses the usefulness of a positive control group with a known immunosuppressant. Outside comments have questioned the need to perform a positive control group with every study, the numbers of animals required for this control group, and have recommended that a laboratory only need to perform this test once every six months.

EPA requests the Panel's recommendation concerning the frequency of performing a positive control with the immunotoxicity study. In addition, EPA requests that the Panel recommend the numbers of animals needed in the positive control groups.

SAP RECOMMENDATION

With regard to Issue 7, use of positive controls, the SAP felt that there was no compelling need to run a positive assay control each time an experiment was performed. Including this control every six months or when new reagents are titrated would be sufficient. The SAP recommends that the number of animals utilized as positive controls be at least 8 per group.

OTHER COMMENTS BY SAP

In addition to these specific issues, a number of other comments were made by SAP. The issue of extra animals to serve as a source of lymphocytes was discussed. The SAP felt that the spleen could serve both as a source of cells for phenotypic analyses as well as histopathology, especially if the rat were used as the test system. The SAP believes that histopathological analysis can be performed on animals that have been immunized with sRBC because published studies showing that the analysis is not affected and other toxicologic pathology evaluations are performed on immunized animals e.g. primates with TB, dogs with rabies.

The final issue discussed concerned the inclusion of the natural killer (NK) assay in the routine screen. The SAP felt that neither the AFC nor phenotyping studies assessed innate immune function as the NK assay does. Furthermore, evidence exists that this parameter can be modulated in the absence of other functional changes. In addition, from a risk assessment perspective, NK cell function has been shown to be important in resistance to certain viral infections and neoplasias in humans. Although the SAP recognizes that the normal baseline responses are low and may not allow much downward movement if immune suppression is suspected, dose-response modulation of this assay has been demonstrated. It is for these reasons that the SAP recommends that measurement of NK cell function be included in the screen.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

_____
LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

and

SCIENCE ADVISORY BOARD

JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency in Connection with the

Domestic Animal Safety Guidelines under the Health Effects Test Guidelines OPPTS 870.7200 Domestic Animal Safety.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues regarding the Domestic Animal Safety Guidelines. The review was conducted in an open meeting held in Arlington, Virginia, on October 29, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Marion W. Anders (University of Rochester Medical Center); Dr. Charles C. Capen (Ohio State University); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. William Keller (Food and Drug Administration); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); (Dr. Bernard Weiss (University of Rochester).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

DOMESTIC ANIMAL SAFETY GUIDELINES (OPPTS 870.7200):

EPA's Issues for the SAP's Consideration with Their Recommendations

QUESTION 1: VEHICLE VS. NEGATIVE CONTROL

The draft Guidelines propose using a vehicle control at 5X the recommended dose. The question for the SAP is whether an untreated or a vehicle-treated control should be recommended for these studies.

SAP RECOMMENDATION

Ideally both vehicle and untreated controls should be part of the study. Vehicle control would be particularly important for products labeled for continuous or intermittent long-term use. Untreated controls would be more important if the group size were large enough to see untreated vs vehicle control differences. Unusual or extreme approaches to delivery of 5X the vehicle may not result in useful toxicity information. If the active ingredient has already been thoroughly studied more emphasis might be placed on the vehicle. If cats are on the label more emphasis needs to be placed on characterizing vehicle toxicity. Both types of controls are useful.

QUESTION 2: TESTING OF ADULT AND JUVENILE ANIMALS

The draft Guidelines propose that both adult and juvenile dogs and cats should be tested if the label states that a product may be used in both age groups. Some registrants have argued that testing in juveniles is a worse-case scenario and the results in this age group can be extrapolated to adults.

The question for the SAP is whether young and adult animals should be tested if a product is labeled for use in both age groups or if testing in young animals is sufficient to predict safety in adults.

SAP RECOMMENDATION

There is adequate literature supporting age-differences. If a product is specifically labelled for juvenile animals then it should be tested in that age-group and vice versa. The standard test should be done in both adults and juveniles. In limited circumstances the juvenile animal data might be sufficient. For instance for minor uses or when active ingredients are toxicologically well characterized.

The SAP suggests that the Agency consider the issue of the need for special labelling to protect pregnant and lactating animals. It was not clear to the SAP if any precautions for such animals are now in place.

QUESTION 3: DURATION OF TREATMENT FOR FLEA COLLARS

Most flea collars are labeled for use for an extended period of time, such as five to six months. However, the majority of the active ingredient is released within the first month or so after application to the animal. The draft Guidelines do not specifically address how long the animals should wear collars in the domestic animal safety studies.

The question for the SAP is what should be the duration of treatment for domestic animal safety studies involving flea collars.

SAP RECOMMENDATION

The ideal duration would be life-time but that is not feasable. One month might be sufficient for well-characterized products. For new chemical entities or collar formulations, a longer exposure time might be needed. Several months duration exposure would be reasonable for pesticide products that are not well characterized.

QUESTION 4: MARGIN OF SAFETY REQUIRED FOR REGISTRATION OF PESTICIDES

The draft guidelines suggest a 5X margin of safety for pet products. Some discussion has ensued on whether an observation of clinical signs of toxicity at the 5X dose level would prevent registration of the product in question.

The question for the SAP is whether pet pesticide products should have a 5X margin of safety, regardless of the toxicity endpoints, or if there is latitude for recommending registration of products with less than 5X margin of safety.

SAP RECOMMENDATION

If the determination is whether to register or not then some latitude is in order. A 5X limit might be considered for products used as preventives on healthy animals. The question of "why treat the animal"? should be asked. If the answer is compelling then some risk is warranted. If other effective alternatives are available then the need for registration is lessened. There should be some latitude, but if the need is great and the risk clearly characterized and acceptable then less than 5X would be acceptable. The SAP is concerned about the use of these products in pregnant and lactating animals. Again, is the Agency considering this issue?

QUESTION 5: NUMBER OF ANIMALS PER GROUP

The draft guidelines suggest that six animals/sex/group be tested. Several public comments have recommended that this number be changed to four animals/sex/group. The number suggested in the draft guidelines was based on the requirements of the Center for Veterinary Medicine, Food and Drug Administration, in their testing of veterinary drugs.

The question for the SAP is whether the number of animals/sex/group should remain as six or be reduced.

SAP RECOMMENDATION

Four or six animals per group is very small when characterizing margins of safety. Sufficient justification exists for a variety of numbers/group. Four animals/sex/group is more commonly used. The study requirements at CVM are flexible. For new chemical entities the need for more data would be consistent with larger group sizes. This is important since no clinical trials are required and FDA regulations require these studies for approval. If data are available from subchronic dog studies the need for six animals would

be lessened. Less background information is usually available for cats so that 6 cats/sex/dose would be needed. Some recognition of the facilities and staff limitations of industry needs to be made in requiring larger number of animals/dose. These data requirements should be justified.

SAP's initial response to EPA's presentation emphasized the need for harmonization and consistency across similar products and regulatory agencies both nationally and internationally. International factors such as different backgrounds and mandates for regulatory agencies were noted. Marketing factors such as the opportunity to select the regulatory agency for a product by formulating a product to fall within EPA vs FDA's area, and claiming superiority of one product over another within a market area based on whether the product is regulated by EPA vs FDA were noted as adverse consequences of a lack of harmonized requirements.

Other SAP comments included the recommendation that this guideline be renamed the "Companion Animal Guideline" and that guideines for other domestic animals be developed and harmonized during the development of these new guidelines with CVM's Guideline for Target Animal Safety.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

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LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

and

SCIENCE ADVISORY BOARD

JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency in Connection with the Comparison of the Effects of Chemicals with Combined Perinatal and Adult Exposure vs Adult Only Exposure in Carcinogenesis Bioassays.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues regarding the Comparison of the Effects of Chemicals with Combined Perinatal and Adult Exposure vs Adult Only Exposure in Carcinogenesis Bioassays. The review was conducted in an open meeting held in Arlington, Virginia, on October 29, 1996. The meeting was chaired by Dr. Marion W. Anders. Other panel members present were: Dr. Ernest E. McConnell (recuse); Dr. Charles C. Capen (Ohio State University); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); (Dr. Bernard Weiss (University of Rochester).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

IN UTERO BIOASSAY

EPA's Questions for the SAP's Consideration with Their Recommendations

QUESTION 1: IS IN UTERO TESTING ROUTINELY NECESSARY?

Would the members please comment on the Agency's conclusion that existing data do not support the routine requirement of an in utero carcinogenesis bioassay? Comment on the Agency's conclusion that existing data do not support the routine requirements of an in utero carcinogenesis bioassay?

SAP RECOMMENDATION

The SAP agrees with the Agency's conclusion. The present data in the literature supports the Agency's position, although the data set is not particularly robust. There is no convincing data that perinatal exposure would increase the sensitivity of the standard carcinogenesis bioassay.

QUESTION 2: WHAT FACTORS SHOULD EPA CONSIDER IN REQUIRING IN UTERO TESTING?

The FDA employs a set of criteria in assessing the requirement of an in utero carcinogenesis bioassay. The Agency requests from the members of the SAP suggestions for general factors to consider in requiring an in utero carcinogenesis bioassay for pesticides. The Agency requests from members of the SAP suggestions for a set of criteria to serve as triggers for requiring an in utero carcinogenesis bioassay for pesticides.

SAP RECOMMENDATION

The criteria employed by the Toxicology Branches of the Food Safety and Applied Nutrition, U.S. FDA for considering chemicals as candidates for testing in a perinatal carcinogenicity study (1982 Redbook) are considered by the SAP as reasonable starting points. However, concern was expressed about one criterion, viz. "compounds with reproductive toxicity or teratogenic activity." It was pointed out that reproductive toxicity usually is a variable independent of carcinogenic potential of a compound, suggesting that this may not be a useful criterion for triggering an in utero carcinogenesis bioassay. Other criteria used by the FDA include: (a) compounds whose lowest "effect" level is less than 200-times the expected human exposure; (b) compounds which are used as non-nutritive additives and whose exposure exceeds 0.25 μg/kg/day; (c) compounds which are considered as nutritive additives; (d) any compound with data indicating differences in affected organs in utero studies vs. non-in utero studies which require further investigation; and (e) compounds with other data (reproductive and developmental toxicity) indicating a need for in utero exposure. The SAP finds that the last two criteria (d and e) are especially applicable to the Agency.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

_____
LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

and

SCIENCE ADVISORY BOARD

JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency in Connection with the Health Effects Test Guidelines OPPTS 870.3700 Prenatal Developmental Toxicity Study and the Health Effects Test Guidelines OPPTS 870.3800 Reproduction and Fertility Effects.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues regarding the Developmental and Reproduction Guidelines. The review was conducted in an open meeting held in Arlington, Virginia, on October 30, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were Dr. Marion W. Anders (University of Rochester Medical Center); Dr. Charles C. Capen (Ohio State University); Dr. Robert E. Chapin (NIEHS); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); Dr. Mary Anna Thrall (Colorado State University); (Dr. Bernard Weiss (University of Rochester); Dr. Barry Zirkin (Johns Hopkins University).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

Oral statements were received from:
Dr. Abe Tobia, American Crop Protection Association
Dr. Mark Cukierski, Merck Research Laboratories
Dr. Dana L. Shuey, Rohm & Haas Company
Dr. David L. Eisenbrandt, Dow Chemical Company

DEVELOPMENTAL TOXICITY TESTING GUIDELINES (OPPTS 870.3700)

EPA's Issues for the SAP's Consideration with Their Recommendations

SAP'S GENERAL COMMENTS ON THE DEVELOPMENTAL GUIDELINES

The Panel strongly supports the development and quick acceptance and application of these revised Guidelines, taking into account the suggestions below. These new Guidelines include the evaluation of a large number of endpoints which lack a readily accessible and widely available database. Because of the "speed of advance" in our understanding for many of these areas, we strongly recommend that these Guidelines become an iterative process: collect the data, after a period of 1-3 years or so, convene a working group to evaluate whether these data are truly providing the information desired with the necessary statistical power, and then amend the Guidelines appropriately. The Workgroup should consist of knowledgeable experts who could appropriately and transparently render judgements about the value of the data relative to the costs associated with the collection, and with the legal requirements constraining the Agency. After these Workgroup deliberations, future presentations to the Scientific Advisory Panel should include a summary of the data used for the assessment, a clear statement of the criteria, and the Agency's decision based on these criteria and database. Only with such information before it can the Panel render the best scientific judgements. The process for both the 1996 and 1993 guideline meetings (presenting decisions in the absence of virtually any supporting data) left the Panel limited with information necessary to render appropriate judgements. We hope that the next time these Guidelines come before the SAP, that the Panel will be presented with the appropriate data, the selection criteria used by the Agency or workgroup, and the resulting decision.

Provided below are the questions that EPA presented to the SAP along with the SAP's recommendations.

QUESTION 1: DOSING RABBITS LATE IN GESTATION

In the proposed guideline, dosing of the dams has been extended to include the period from implantation to approximately the end of gestation, with the option of dosing from mating to termination. No distinction is made between rodent and nonrodent (rabbit) species in the implementation of this dosing regime. Several commenters have expressed concern that for rabbits, which tend to abort their litters with higher frequency than rats, dosing in late gestation may induce abortions and interfere with the study results. The Agency has no evidence to suggest that this supposition has been tested in any manner.

What is the experience and opinion of the Panel regarding dosing rabbits in late gestation?

SAP RECOMMENDATION

The collective opinions support the concept of dosing rabbits in late gestation to capture potential effects on sexual development. The re-evaluation of these data in a couple of years should include an assessment of the severity of the postulated problems with litter resorptions/abortions.

QUESTION 2 IS BIAS OF CONCERN, AND SHOULD EVALUATIONS BE DONE "BLIND" FOR DOSE?

The guideline recommends that the evaluation of the dams during cesarean section and the subsequent fetal analysis be conducted without knowledge of treatment group in order to minimize bias. Several commenters have stated their disagreement with this requirement, arguing that bias does not occur and/or that such "blind" evaluation would be difficult to implement.

Does the Panel agree that bias should be of concern and that the evaluation of dams at c-section and the evaluation of fetuses for external, soft tissue, and skeletal effects should be conducted without knowledge of treatment group?

SAP RECOMMENDATION

The Panel strongly supports the concept of performing these evaluations blind for dose. We recognize that this does not imply a bias in registrant studies, but reflects the mainly subjective nature of many of these determinations. Ignorance of dose level when evaluations occur will ensure that the best data are collected.

QUESTION 3 SHOULD FETAL CARTILAGE BE STAINED?

In the latest proposed revision to this guideline, the evaluation of cartilage is not mandatory, but the guideline specifies that the Agency prefers that both bone and cartilage be evaluated in the process of skeletal evaluation. The methodology is not specified, in order to allow flexibility in laboratory procedures, although generally, double staining of fetuses with alizarin red S and alcian blue has been assumed by industry.

Does the Panel agree that fetal cartilage development should be assessed in order to provide a more complete evaluation of skeletal development?

SAP RECOMMENDATION

The Panel strongly supports the evaluation of fetal cartilage concurrent with bone evaluations. This will not only provide additional new information, but the cartilage findings should support and confirm any bone findings. The Panel supports the Agency's

flexibility in allowing registrants to decide which method will best suit each registrant in terms of appropriate data of acceptable quality to present to the Agency.

REPRODUCTIVE AND FERTILITY TESTING GUIDELINES (OPPTS 870.2800)

EPA's Issues for the SAP's Consideration with Their Recommendations

SAP'S GENERAL COMMENTS ON THE REPRODUCTIVE AND FERTILITY GUIDELINES

As with the Developmental Guidelines, the Panel was without most of the data necessary to effectively answer the questions posed to it. The SAP recommends the adoption of these Guidelines without further Panel review, subject to the changes suggested below.

Provided below are the questions that EPA presented to the SAP along with the SAP's recommendations.

QUESTION 1: TEN-WEEK TREATMENT PERIOD FOR THE P GENERATION

The current and proposed guidelines require a 10-week treatment period for P animals (male and female) prior to mating, and a subsequent 10-week treatment period for selected F1 animals after weaning. Additionally, the proposed revisions to the reproduction testing guidelines for OECD (guideline 416) and to the FDA Redbook guideline for testing of food additives, require the 10-week treatment period for males. On the other hand, the International Committee on Harmonization (ICH) Harmonized Tripartite Guideline for the Detection of Toxicity to Reproduction for Medicinal Products, which was finalized in June 1993 with the approval of FDA and OECD, state that a 4-week treatment period for P males, along with a 10-week treatment period for F1 males, is sufficient to evaluate the potential for an effect on male reproductive capacity. It is, however, the contention of Agency scientists that, because this study is a first tier screening tool for pesticides and toxic substances, the males on a two-generation reproduction study in rats should be exposed for the duration of the entire period of spermatogenesis (approximately 70 days) in order to adequately assess toxic effects.

Does the Panel agree with a 10-week treatment period for both P and F1 males?

SAP RECOMMENDATION

Yes, the Panel agrees with the recommendation for a 10 week P treatment period.

There was minority support for a 4 week P exposure; this should be included in the re-evaluation in a few years.

QUESTION 2 ASSESSMENT OF ALL ADULTS ON STUDY FOR SPERM ENDPOINTS AND ESTROUS CYCLICITY

Evaluation of estrous cyclicity and sperm measures (count, morphology, motility) are required for all adult animals in the proposed guideline. It is intended that by assessing all animals on the study, the possibility of making correlations between these endpoints and other measures such as fertility and postmortem findings will be possible. Several comments have been received that recommend a reduction of the number of animals assessed in each generation, e.g., by random selection of 10-15 animals/sex/group. Although statistically, a 15 animal sample size might be adequate to detect effects within the group, for some variable parameters it might be inadequate. Additionally, it would be difficult to correlate data for specific animals with reproductive effects if those animals were not part of the random sample.

Does the Panel agree that an assessment of all adult animals on study should be performed?

SAP RECOMMENDATION

Yes, the Panel agrees with the concept of evaluating all the adults, at least for the 3 year initial period. However, the Panel remains unconvinced that being able to link functional effects in-life with necropsy data for each adult will significantly change the overall determination of toxicity and effect levels for a given compound. Based on a limited dataset from the NTP, we believe that the statistical power to be gained by going from 10 to 20 rats/group is minimal for some endpoints, and moderate for some other endpoints. The Panel agrees with the concept of collecting sperm and estrous data on all animals in control and high dose groups (with triggered collection of middle and low dose groups depending on finding treatment-related effects) with the proviso that the necessity of this collection will be revisited by the above-mentioned workgroup in 3 years from the instatement of these guidelines.

QUESTION 3: POSTMORTEM SCREEN FOR ADULTS AND OFFSPRING

The sections on the postmortem evaluation of parental animals and offspring have been through several revisions to arrive at the currently proposed version. This includes gross necropsy, organ weight, tissue preservation, and histopathology, as described in pages 6 and 7 of the guideline. Comments and suggestions regarding the number of animals examined and the extent of the evaluation have been received.

Does the Panel agree with the postmortem screen for adults and offspring as proposed, including the sample sizes and the organs selected?

SAP RECOMMENDATION

Based on discussions with experts, and in the absence of supporting data presented by the Agency, we do not see the significant benefit to be gained by performing histopathologic evaluation on more than 10 animals/sex/dose group. We were not convinced that performing histopathologic evaluations on developmental anomalies would provide a significant benefit, relative to the costs associated with that evaluation. Data presented did not convince the Panel that evaluating more than 20 weanlings/sex/dose group would provide significant benefit, and it would be associated with significant logistical problems. In the absence of any supporting data from the Agency showing why the proposed numbers of 3/sex/litter were chosen, we believe that 1/sex/litter should be sufficient. Also, no data were presented to support the inclusion of the ovarian follicle counts as proposed in these Guidelines. On the contrary, even the authors of this method (Mattison and Plowchalk) do not believe that this is an appropriate screening technique. Thus the Panel recommends that the Agency avoid ovary counts. However,if it is used, we suggest evaluating only control and high dose F1 females, 10/group, 20 sections/female, including primordial and antral/pre-antral designations. This endpoint should be revisited in 3 years.

QUESTION 4: OPTION FOR THE EVALUATION OF MATURATIONAL LANDMARKS

The OECD proposed test guideline 416 recommends optional maturational landmark or functional testing procedures. In Item 34 of that guideline, it states that

> "Other physical landmarks and/or functional investigations such as testing of reflex ontogeny may be a good supplementation, and should preferably be related to sexual maturation. The performance of functional tests in the F1 offspring after weaning is recommended when separate studies on neurodevelopmental toxicity are not considered. Functional tests may, however, be omitted in groups that otherwise reveal clear signs of adverse effects (e.g., significant decrease in weight gain, etc.)"

The Agency has elected not to include maturational landmarks or functional testing in the OPPTS 870.3800 standard two-generation reproductive toxicity guideline. These endpoints are currently addressed in the developmental neurotoxicity testing guideline; however, the value of including these endpoints in a standard two-generation screen is recognized.

Does the Panel agree that the OPPTS guideline should not include an option for the

evaluation of maturational landmarks or functional testing?

SAP RECOMMENDATION

The SAP agrees with the Agency that neurodevelopmental landmarks (pinna detachment, etc.) need not be evaluated in these reproduction studies.

GENERAL COMMENTS AND RECOMMENDATIONS

The discussion subsequent to the Agency's presentation included comments about the difficulty of late-gestation rabbit dosing. The Panel strongly agrees that sperm morphology is an important endpoint that can provide useful data (both stand-alone, and correlative), despite a comment made to the contrary during the discussion period. The Panel also agrees that it will be useful to collect the organ weights specified in these new Guidelines, even though some of these organ weights duplicate to some degree some data collected in previous studies. We applaud the Agency's flexibility on culling, and would like to propose that the Agency be a focal point for a series of prospective studies on the effect of culling on study outcomes. The field has long needed such a study, which could be collaborative with other Government agencies/institutes, contracted, or partnered with industry. Finally, the recommendations made above are made without all of the details of the legislative mandates driving many of the Agency's current decisions. Where the SAP recommendations conflict with these legislative mandates, we respect these Congressional imperatives, but would urge the Agency to clearly articulate in some form and venue the necessity for the inclusion of any significantly burdensome and scientifically unproven or uncertain endpoints. We repeat our request for the formation of a working group to evaluate these new data collected under these Guideline revisions and make public the decisions reached as a result of these evaluations.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

_____
LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

and

SCIENCE ADVISORY BOARD

JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency to Discuss and Evaluate the Weight-of-Evidence for Vinclozolin with Particular Reference to its Potential for Developmental and Reproductive Toxicity.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues Relating to Vinclozolin and its Potential for Developmental and Reproductive Toxicity. The review was conducted in an open meeting held in Arlington, Virginia, on October 29, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Marion W. Anders (University of Rochester Medical Center); Dr. Charles C. Capen (Ohio State University)(recuse); Dr. Robert E. Chapin (NIEHS); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); Dr. Mary Anna Thrall (Colorado State University); (Dr. Bernard Weiss (University of Rochester); Dr. Barry Zirkin (Johns Hopkins University).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

Oral statements were received from:
Dr. Abraham Tobia, BASF Corporation
Dr. Jurgen Hellwig, BASF Corporation
Dr. Bennard van Ravenzwaay, BASF Corporation

Written statemens were received from:
Dr. Abraham Tobia, BASF Corporation
Dr. Jurgen Hellwig, BASF Corporation
Dr. Bennard Van Ravenzwaay, BASF Corporation
K. A. Davidson, Chemical Health Evaluation Group
J. Francis, Chemical Health Evaluation Group

VINCLOZOLIN DEVELOPMENTAL AND REPRODUCTIVE EFFECTS

EPA's Questions for the SAP's Consideration with Their Recommendations

QUESTION 1: EPA'S POSITION ON AGD

Does the Panel agree with the Agency's position that decreased anogenital distance (AGD) in rats observed in developmental and perinatal studies with vinclozolin is a toxicologically meaningful indicator of potential androgen deprivation during human development with vinclozolin exposure?

SAP RECOMMENDATION

The AGD data can only be considered as part of a corpus of data that include the androgen receptor binding data and other reproductive-development endpoints. By itself, a change in AGD suggests an effect and possibly a mechanism. Together with these other data presented on vinclozolin and its metabolites on the androgen receptor and the increase in areolas, prostate and seminal vesicle weights and male sex organ anomalies at higher doses, vinclozolin demonstrates several toxicological indicators of potential androgen deprivation. For vinclozolin, **because of these other data**, the AGD change is believed to be the most sensitive sentinel endpoint.

QUESTION 2: AGENCY'S INTENTION TO USE ABSOLUTE AGD

Does the Panel agree with the Agency's intention to use absolute AGD, rather than AGD/body weight (AGD index), as the critical measure for AGD for the purpose of evaluating potential risk to humans?

SAP RECOMMENDATION

The panel was given neither data nor information before the meeting on this issue. Without adequate time to evaluate these data, the Panel has to assume that the summary information presented by both the industry representatives and the Agency are valid. In conjunction with a limited NTP data set, the panel agrees that absolute AGD has scientific validity and should be used.

QUESTION 3: TOXICOLOGICAL SIGNIFICANCE OF DEVELOPMENTAL EFFECTS OTHER THAN DECREASED AGD

The Panel is asked to comment on the potential toxicological significance of developmental effects other than decreased AGD in rats that were also observed in the developmental and perinatal studies on vinclozolin, and comment on which of these findings would be sufficient for quantifying potential developmental risk to humans? The other developmental effects were: (a) nipple/areolas development in males at ≥6 mg/kg/day, (b) dose related nominal decreases in ventral prostrate weight at ≥12 mg/kg/day; (c) decreased ventral prostate and seminal vesicle weight at ≥25 mg/kg/day, decreased sperm count and production at ≥50 mg/kg/day; and, (d) severe developmental effects on male sex organs at 100 mg/kg/day, such as hypospadias.

SAP RECOMMENDATION

The data on vinclozolin present a clear picture of an antiandrogic chemical. These data presented from both industry representatives and the Agency suggest that AGD is the most sensitive indicator of this toxic action with the nipple/areolas being the next best indicator. However, the Panel concludes that the other developmental effects noted above are also important for consideration for vinclozolin as a potential developmental toxicant.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

_____

LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

and

SCIENCE ADVISORY BOARD

JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency to Discuss and Evaluate the Weight-of-Evidence for Vinclozolin with Particular Reference to its Carcinogenic Potential.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues Relating to Vinclozolin and its Carcinogenic Potential. The review was conducted in an open meeting held in Arlington, Virginia, on October 30, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Marion W. Anders (University of Rochester Medical Center); Dr. Charles C. Capen (Ohio State University) (recuse); Dr. Robert E. Chapin (NIEHS); Dr. Sam Cohen (University of Nebraska); Dr. Philip Guzelian (University of Colorado); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Albert E. Munsen (NIOSH); Dr. Steve Schrader (NIOSH); Dr. Peter Thomas (IIT Research Institute); Dr. Mary Anne Thrall (Colorado State University); (Dr. Bernard Weiss (University of Rochester); Dr. Barry Zirkin (Johns Hopkins University).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

Oral statements were received from:
Dr. Jurgen Hellwig, BASF Corporation
Dr. Bennard van Ravenzwaay, BASF Corporation

THE CARCINOGENICITY OF VINCLOZOLIN

EPA's Questions for the SAP's Consideration with Their Recommendations

Dr. David Anderson from the Agency presented a review of the carcinogenicity data and issues on vinclozolin and he and Dr. Karl Baetcke responded to questions. Three BASF representatives presented information based on a re-evaluation of the pathology. Discussion ensued regarding the nature of the histopathology interpretation of the lesions in rats, possible antiandrogenic activity or other mechanisms involved in generation of these lesions, and the relevance of the findings in rats to humans.

Provided below are the questions that the Agency posed to the Panel along with the Panel's recommendations.

QUESTION 1: DOES VINCLOZOLIN HAVE A CARCINOGENIC POTENTIAL?

Does the Panel agree with the Agency's conclusion that vinclozolin has carcinogenic potential in both sexes of rats and that the tumors are treatment-related and relevant for evaluation of potential risk to humans? The tumors with statistically significantly increased incidences that support this conclusion were observed at a dose level greater than or equal to 500 ppm (23 mg/kg/day for males and 30 mg/kg/day for females) and were testicular Leydig cell adenomas and prostate adenomas in males and benign ovarian sex cord stromal tumors in females.

SAP RECOMMENDATION

It is not clear that vinclozolin is carcinogenic in either sex of the rat. The target tissues (ovary, testes, prostate) are endocrine controlled, with a relatively high incidence of proliferative lesions in controls. Review of the pathology of the ovary and prostate, using well-defined criteria, showed mostly hyperplasia, with few adenomas (benign tumors) and no malignancies. The testes showed only benign interstitial cell tumors, a common occurrence in this strain of rats. Based on the re-evaluation of the pathology, there was a significant increase only in testicular Leydig cell adenomas. Based on these data, it is far from established that vinclozolin is carcinogenic to the rat. It is not ruled out, however. In addition, there is little concern for mutagenicity as expressed by the Agency reviews.

QUESTION 2: CAUSE OF THE ANTIANDROGENIC PROPERTIES

Does the Panel agree with the Agency's conclusion that the antiandrogenic properties of vinclozolin are most likely responsible for the increased incidence of testicular Leydig cell adenomas observed in the long-term studies in Wistar rats?

SAP RECOMMENDATION

The antiandrogenic effects of vinclozolin were assessed primarily by analogy to similar

effects by flutamide and other well studied structurally related antiandrogens. In rats, vinclozolin showed increased LH and serum testosterone levels, effects which can be produced by a variety of mechanisms. Data showing specific inhibition of the testosterone receptor by metabolites M1 and M2 were presented in the previous session, and this, together with data showing reduced ventral prostate weight and anogenital distance, leaves little doubt that vinclozolin has antiandrogenic action. The increased testosterone levels are likely the factors producing Leydig cell hyperplasia/tumors, as has been well studied in rats in other models.

QUESTION 3: ADEQUACY OF DATA TO SUPPORT AN ANTIANDROGENIC MODE

Does the Panel agree with the Agency's conclusion that the data have not been sufficiently developed to support an antiandrogenic mode of action for other tumors observed (prostate adenomas and ovarian sex cord adenomas) in carcinogenicity or chronic studies on vinclozolin?

SAP RECOMMENDATION

See above 1 and 2.

QUESTION 4: VINCLOZOLIN CANCER CLASSIFICATION

Would the Panel please comment on the Agency's carcinogenicity assessment and classification of vinclozolin (Group B2, probable human carcinogen)? The carcinogenicity classification was based on antiandrogen/hormone related testicular Leydig cell tumors and possible, but unproven antiandrogen/hormone related benign prostate tumors and benign ovarian sex cord tumors (all seen in rats).

SAP RECOMMENDATION

Based on (1), we would consider the possibility that vinclozolin is a carcinogen in rats or mice, but the evidence for this is not compelling. The Panel believes that the classification of vinclozolin using the new guidelines would be "not likely to be a carcinogenic hazard to humans".

QUESTION 5: APPROPRIATENESS OF THE METHOD OF QUANTIFICATION

Would the panel please comment on the appropriateness of the method of quantification? The method of quantification recommended by the Agency for potential carcinogenic risk to

humans was a non-linear model, MOE (NOEL/(chronic dietary exposure)) approach based on an NOEL for non-neoplastic anti-androgenic related effects.

SAP RECOMMENDATION

The most appropriate method of risk quantification is on a non-linear model, MOE approach based on a NOEL for non-neoplastic effects.

FOR THE CHAIRMAN:

` Certified as an accurate report of findings:

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LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

and

SCIENCE ADVISORY BOARD

JOINT MEETING ON GUIDELINE ISSUES

A Set of Scientific Issues Being Considered by the Agency to Discuss and Evaluate the Weight-of-Evidence for Alachlor with Particular Reference to its Carcinogenic Potential.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) and Science Advisory Board have completed their joint review of a set of scientific issues Relating to Alachlor and its Carcinogenic Potential. The review was conducted in an open meeting held in Arlington, Virginia, on October 30, 1996. The meeting was chaired by Dr. Ernest E. McConnell. Other panel members present were: Dr. Marion W. Anders - recused (University of Rochester Medical Center); Dr. Charles C. Capen -recused (Ohio State University); Dr. Sam Cohen (University of Nebraska); Dr. Ronald Kendall (Clemson University); Dr. Michele Medinsky (Chemical Industry Institute of Toxicology); Dr. Harihara Mehendale (Northeast Louisiana University); Dr. Genevieve M. Matanoski (Johns Hopkins University); Dr. Bernard Weiss (University of Rochester); Dr. Barry Zirkin (Johns Hopkins University).

Public Notice of the meeting was published in the Federal Register on August 28, 1996.

Oral statements were received from:
Dr. Ian C. Munro, Monsanto
Dr. James A. Swenberg, Monsanto
Dr. Gary M. Williams, Monsanto
Dr. Alan Wilson, Monsanto

Written statements wre received from:
Dr. Ian C. Munro, Monsanto
Dr. James A. Swenberg, Monsanto
Monsanto Corporation

THE CARCINOGENICITY OF ALACHLOR

EPA's Questions for the SAP's Consideration with Their Recommendations

A review of the carcinogenicity issues for Alachlor was presented by Drs. Dapson and McMahon of the EPA. Summaries of relevant data were presented, including carcinogenicity studies, additional mutagenicity studies, mechanistic data, additional metabolism studies and toxicity data for a structurally related chemical, Butachlor. EPA concluded that the mouse lung tumor data were inappropriate for human risk assessment. Likewise EPA concluded that the thyroid tumors were the result of a rat specific, high dose hormonally induced mechanism and not relevant for human risk assessment. Rat nasal tumors were suggested to be the result of a biochemical mechanism more prevalent in rats than other species including humans. Stomach tumors were suggested as the most appropriate tumor for comparison to humans, with similarities to known human pathological conditions. A MOE approach was recommended for estimation of human risk.

Drs. James Swenberg, Gary Williams, and Ian Munro, representing Monsanto as experts, provided public comments. They noted that: alachlor is not carcinogenic in the mouse; the weight of evidence fully supports the view that alachlor is not genotoxic in mammalian systems; and therefore alachlor does not pose a significant risk to humans. Since tumors of the stomach and thyroid only occurred in the rat and at a dose that exceeded the MTD. Commenters noted that these tumors were not appropriate for consideration for risk assessment. The nasal tumor data were thought to be most relevant, with the mode of action driving nasal tumor formation in rats related to species-specific distribution, metabolism, and cytotoxicity. Further support for this conclusion comes from negative epidemiology studies in workers exposed to alachlor.

The Agency specifically requests comments from the SAP regarding our assessment of the weight-of-evidence relating to the proposed mechanism(s) for induction of nasal, gastric, and thyroid tumors by treatment of Long-Evans rats with Alachlor in the diet; specifically:

QUESTION 1: SIGNIFICANCE OF RAT NASAL TUMORIGENESIS

The proposed mode of action for nasal turbinate tumor induction is based on evidence demonstrating biotransformation of alachlor to a reactive metabolite, with binding of this metabolite to cellular protein, eventual cell death, and subsequent neoplasia. While rats and humans are recognized to possess the same biotransformation pathways involved in production of this metabolite of alachlor, it is also recognized that the activity of these pathways is substantially greater in the rat compared to the human, and that rats also demonstrate unique localization of this metabolite in the nasal turbinates compared to other species. Therefore, is the proposed mechanism for rat nasal tumorigenesis relevant for human cancer risk assessment?

SAP RECOMMENDATION

It is not clear why adenocarcinomas occurred rather than the usual squamous cell carcinomas occurring in this region. The nasal tumors are the endpoint most appropriate for a cancer risk assessment since they occurred at doses below the MTD. The appropriateness of using the MOE approach is dependent on convincing data that alachlor metabolites are nongenotoxic in rat nose. Numerous genotoxicity studies on Alachlor itself have been conducted which indicate it is nongenotoxic. The specific mechanism proposed for tumor formation involves biotransformation, translocation, and subsequent metabolic activation in situ in rat nasal tissue to a reactive metabolite. The genotoxicity of precursors to this metabolite are weakly genotoxic in bacterial mutagenesis assays. The strength of the evidence for the formation of very low levels of DNA adducts after alachlor administration should be commented on specifically by the EPA. DNA adducts would provide indirect support for the genotoxicity of Alachlor.

The strength of evidence for these data on human metabolism of Alachlor by human nasal tissue should also be addressed. Interspecies differences in bioactivation of alachlor appear to be critical as biotransformation is the key step in initiating the cytotoxicity and tumor response. Autoradiography data demonstrating localization of alachlor metabolites only in rat nasal tissue and not mouse or monkey is suggestive of interspecies differences in formation of a reactive product that is retained by nasal tissues, providing indirect evidence for the role of metabolic activation in the carcinogenic process. Thus, because bioactivation is thought to play a key role in the mechanism for nasal tumor formation, the evidence that bioactivation in humans occurs at significantly lower rates should be compelling. The presence of these nasal enzymes in humans is indicative of a qualitative rather than quantitative response, suggesting that the shape of the dose response curve is very different across species rather than the mechanism for production of nasal tumors being not relevant for humans. The analogy to phenacetin is also noteworthy. Phenacetin also produces nasal tumors in rats. However, in humans it is carcinogenic to the lower urinary tract (urothelium), but only at extremely high doses (kg, total ingestion). Thus, although alachlor cannot completely be excluded from having activity in humans, it is highly likely that if it occurs at all, it would only occur at doses far in excess of exposure levels. Therefore, an MOE approach to human risk assessment of alachlor is appropriate.

Data presented by Monsanto showed that there was a `30-fold higher metabolism of alachlor in the rat compared to the mouse. Since the rat does respond with nasal cancer and the mouse does not, this difference in metabolism is thought to be the critical mechanism. This rationale is extended to the human, where several thousand-fold lower activity in the metabolism of alachlor was found. When asked if the intermediate metabolite just beyond the most ratelimiting step in the metabolism causes nasal tumors in the mouse, the registrants responded by saying that limitation in metabolism is not the only factor for lack of tumorigenic response in the mouse. Therefore, the limitation in the metabolism in the mouse may not be the real reason for the lack of tumorigenic response in the mouse. If this is accurate, then the argument that limitation in the metabolism of

alachlor in the human precludes alachlor being considered as a human carcinogen can not be supported.

QUESTION 2: MODE OF ACTION FOR THYROID TUMOR

The proposed mode of action for the thyroid tumor is said to be the result of induction of hepatic glucuronyl transferase with subsequent decrease in circulating T3 and T4, a subsequent increase in TSH, and eventual hyperplastic response of the thyroid. Does the panel agree that interpretation of these data support the proposed mechanism for thyroid tumor induction?

SAP RECOMMENDATION

The panel agrees that the interpretation of the data support a hormonally induced mechanism for the formation of thyroid tumors. This mechanism may be relevant for humans. However, since the tumors occurred only at doses in excess of the MTD, their usefulness for risk assessment is questioned.

QUESTION 3: NON-GENOTOXIC MECHANISM

The mode of action for the stomach tumor is said to be the consequence of a direct contact effect via a non-genotoxic mechanism resulting from an indirect response to a change in pH. Does the panel agree that the interpretation of these data support the proposed mechanism?

SAP RECOMMENDATION

The stomach tumors occurred only at doses in excess of the MTD and thus are probably not relevant to humans. With regard to the mechanism of tumor formation, more details regarding the tumor types detected should be provided. For example, although detailed studies of stomach tumors after administration of Butachlor were reported, similar detailed immunohistochemical analyses have not been reported for alachlor. Evidence was presented that the carcinomas resulting from alachlor were examined to prove that they were carcinoids, not adenocarcinomas or gastric sarcomas, which are unrelated to the proposed gastrin-induced effect.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

LARRY C. DORSEY
Executive Secretary
FIFRA Scientific Advisory Panel

Date: _____