SAP Minutes No. 2005-03

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

A COMPARISON OF THE RESULTS OF STUDIES ON PESTICIDES FROM 1- OR 2-YEAR DOG STUDIES WITH DOG STUDIES OF SHORTER DURATION

MAY 5 AND 6, 2005
FIFRA Scientific Advisory Panel Meeting,
held at the Holiday Inn - Rosslyn at Key Bridge,
Arlington, Virginia

NOTICE

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The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and established under the provisions of FIFRA as amended by the Food Quality Protection Act (FQPA) of 1996. The FIFRA SAP provides advice, information, and recommendations to the Agency 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 FIFRA SAP on an ad hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA 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 Myrta R. Christian, SAP Designated Federal Official, via e-mail at christian.myrta@epa.gov.

In preparing the meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented by the Agency within the structure of the charge.

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Myrta R. Christian, M.S.
Designated Federal Official
FIFRA Scientific Advisory Panel
Date: July 13, 2005

Stephen M. Roberts, Ph.D.
FIFRA SAP, Session Chair
FIFRA Scientific Advisory Panel
Date: July 13, 2005

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
May 5 and 6, 2005

A COMPARISON OF THE RESULTS OF STUDIES ON PESTICIDES FROM 1- OR 2-YEAR DOG STUDIES WITH DOG STUDIES OF SHORTER DURATION

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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 pertaining to a comparison of the results of studies on pesticides from 1- or 2-year dog studies with dog studies of shorter duration. Advance notice of the meeting was published in the *Federal Register* on March 10, 2005. The review was conducted in an open Panel meeting held in Arlington, Virginia, on May 5 and 6, 2005. Dr. Stephen M. Roberts chaired the meeting. Myrta R. Christian served as the Designated Federal Official.

The FIFRA SAP met to consider and review a comparison of results from 1- or 2-year dog studies on pesticides with dog studies of shorter duration. Under the current CFR Part 158 toxicology data requirements, a 13-week and a 1-year non-rodent (dog) study (guidelines 82-1 and 83-1) are required for all food use pesticides and for pesticides with nonfood uses if use of the pesticide product is likely to result in repeated human exposure to the product over a significant portion of the human life-span. Over the last three decades the Agency has received the results of a large number of dog studies in support of the registration of pesticides. The Agency has conducted a retrospective analysis of dog studies that provided the basis for the selection of reference doses (RfDs) in order to determine whether the requirement for both a subchronic and a chronic dog study continues to be justified. The analysis involved a comparison of the results of 13-week studies and 1- or 2-year studies or a comparison of interim data (13-weeks or less) from 1-year dog studies with the data from 1-year in the same study. The Agency solicited comments from the FIFRA Scientific Advisory Panel on this retrospective analysis of the results of dog studies and, specifically, on whether the analysis supports the continuation of the requirement for both subchronic and chronic dog studies or whether consideration should be given to a modification of the current requirements for dog studies. The agenda for this SAP meeting involved an introduction, background, and detailed presentations of the issues related to a comparison of results from 1- or 2-year dog studies on pesticides with dog studies of shorter duration provided by Dr. Karl Baetcke (Health Effects Division, Office of Pesticide Programs, EPA). Dr. Tina Levine (Acting Director, Health Effects Division, Office of Pesticide Programs, EPA) offered opening remarks at the meeting.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This document addresses the information provided and presented at the meeting, especially the response to the Agency's charge.

PUBLIC COMMENTERS

Oral statement was presented as follows:

On behalf of ZEBET - National German Centre for the Documentation and Evaluation of Alternative Methods to Animal Experiments:

Rainer J. Box, Ph.D.

Written statement was provided by or on behalf of the following group:

U.S. Food and Drug Administration

David Jacobson-Kram, Ph.D., DABT, Center for Drug Evaluation and Research, FDA

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

The Panel commended the Agency's effort in conducting this comparison of dog studies and encouraged the Agency to continue their analyses to better inform the Agency's final decision regarding the length of dog studies that is appropriate for reference dose (RfD) determinations. On the basis of the current analysis, the Panel was unable to reach consensus regarding the strength of the scientific evidence supporting elimination of one-year dog studies. Some Panel members thought that the analysis conducted by the Agency was adequate to justify eliminating the requirement for a one-year study in dogs. Other members of the Panel were not ready to support this conclusion, citing concerns regarding the Agency analysis, as discussed in more detail in the responses to individual charge questions. The Panel discussed two possible paths forward while the Agency addresses the limitations in their analyses: 1) retain the one-year dog study and 2) eliminate the one-year dog study but employ an additional uncertainty factor until sufficient confidence to indicate otherwise. In addition, the Panel encouraged the Agency to engage the international scientific community on this issue (e.g., through international meetings working along the lines already pursued by the pharmaceutical regulating agencies) to establish equivalent, internationally harmonized requirements for pesticides.

Even though some Panel members agreed that the general approach taken by the Agency appears adequate to answer the question of whether a 13-week study is sufficient to assess the toxicity of pesticides in dogs for determining an RfD, they identified limitations in the Agency data review. The following recommendations for addressing these limitations were made: 1) increase the robustness of data analysis by including dog study datasets that were not used for the RfD determination, 2) conduct an analysis more representative of a prospective comparison through delineating the 13-week NOAELs (No Observed Adverse Effect Level) and LOAELs (Lowest Observed Adverse Effect

Level) independent of the 1-year study, and establish data review criteria, 3) consider data analysis for separate classes of pesticides, 4) include additional background information on RfD that provides better perspectives for reviewing the Agency position paper, and 5) revise the title of the Agency position paper to better reflect the purpose of the data analysis.

The Agency clearly presented the analysis of data for the 19 chemicals where NOAELs or LOAELs were lower in the chronic dog studies than in the 13-week dog studies. The Panel agreed that the cases where NOAELs or LOAELs appeared to differ between the 13-week and 1-year dog studies detract from the overall conclusions regarding the equivalent value of the 1-year and 13-week dog studies; in addition, these cases serve to identify weaknesses in the existing study designs. However, opinions differed among Panel members regarding the practical significance of a small difference in the NOAEL relative to the 100-fold default uncertainty factor commonly used in calculating the RfD.

The comparison between similar data analysis for pesticides and pharmaceutical products was informative. However, the different objectives of toxicity testing for the two classes of chemicals rendered the comparison of results and conclusions of limited utility to address the questions posed to the Panel. Nevertheless, a harmonizing approach similar to the International Conference on Harmonization data review for pharmaceuticals should be considered for pesticides.

Some Panel members concluded that there was insufficient data for determining whether an additional uncertainty factor should be considered for deriving the RfD in relation to a 13-week dog study duration. It was held by some that the studies used in the RfD determination should be adequate to address the hazard identification (i.e., toxicity endpoints) as well as the dose-response relationship (e.g., NOAEL and LOAEL).

Several Panel members agreed that refinements of the 13-week dog study would add confidence and decrease uncertainty in the calculation of RfDs. Recommendations for refinement were: 1) optimize the study design by using all available data, 2) increase the number of dose groups to reduce the uncertainty associated with the NOAEL and LOAEL determination, 3) collect toxicokinetic data for interspecies comparison (e.g., between rats and dogs), 4) conduct *in vitro* metabolism studies for interspecies extrapolation, and 5) establish triggers for requiring a 1-year dog study.

During the Panel discussions the Agency clarified that the purpose of the analysis as presented was the identification of NOAELs and LOAELs for the derivation of an RfD. This clarification was important to some Panel members for understanding the intent of particular charge questions. In addition, some Panel members suggested that the Agency consider revising the position paper title to avoid the apparent discordance between the title of the Agency's position paper, the approach to data selection, and the Agency's conclusion from the analysis. One suggested title is “A Comparison of the Results from 1- or 2-Year Dog Studies with Dog Studies of Shorter Duration in Cases

where they have been used for the Determination of an RfD."

PANEL DELIBERATIONS AND RESPONSE TO QUESTIONS

The specific issues addressed by the Panel are keyed to the Agency's background documents, references, and the Agency's charge questions.

Questions

Issue 1: Comparison of the results of 13-week and chronic dog studies

OPP has concluded that there is evidence that little qualitative and quantitative value is added by requiring both a 13-week dog study and a 1-year dog study to support the establishment of chronic oral RfDs for pesticide residues.

Question 1

> Please comment on the adequacy of the approach used and the comparisons made regarding the results of dog studies of different durations. OPP would also appreciate recommendations from the Panel on improving the analysis (e.g., figures and tables) or, if needed, discussion of additional analyses that would elucidate the validity of the conclusions made.

Response

Some Panel members agreed that the general approach taken by the Agency appears adequate to answer the question of whether a 13-week study is sufficient to assess the toxicity of pesticides in dogs for determining an RfD. To answer such a question one would typically survey what is known from Agency pesticide submissions and if available, compare the results of that evaluation with what has been seen with other such analyses. Both of these approaches have been included in the Agency analysis.

The two major objectives of using animal studies for risk assessment of pesticides are to determine potential toxicity and to determine a dose below which such an event would not occur. The degree of correlation in the data for both endpoints from 13-week and 1-year studies is far greater than one would have expected and gives credibility to the conclusions of the review. To have a difference in RfDs of less than 1.5X between the two time endpoints is quite impressive and supports the conclusion that 13-week results are adequate for establishing credible RfDs. When studies are conducted multiple times on the same chemical using an identical protocol in the same laboratory, the NOAEL or LOAEL derived from the different studies would be expected to differ to this extent

based on chance alone. In contrast, one would expect essentially a one-to-one correlation with regard to the target tissue as has been demonstrated in this review, i.e. if a change/lesion was observed at 13-weeks, it would also be expected to be found at 1-year, *albeit* one could envision that additional lesions might be observed because of the chronicity of exposure. However, as shown in the German studies, no additional target organs were identified in the chronic dog studies that were not identified in the shorter dog studies or in rodent studies. It was noted that the German review may be problematic as additional supporting documentation because of the possible overlap of studies with those used by the Agency (see below).

The comparison with the German reviews [Gerbracht and Spielmann (1999) and Spielmann and Gerbracht (2001)] may be appropriate and, if so, would give confidence in the Agency results because they essentially arrive at the same conclusion, i.e. that studies shorter than 1-year appear to be adequate and 1-year studies are not needed to establish a credible RfD. However, the German reviews only identify their chemicals by "number" rather than providing the actual chemical name as in the Agency document. Because of the global nature of pesticide manufacturers, it is likely that both the Agency and German reviews are reporting findings from many of the same studies. To understand the extent of corroboration provided by the German studies, it will be important to determine the degree to which they rely upon different studies. If they are using different sets of data, this certainly supports the Agency's position to a greater degree. However, if data are from the same studies, the German results would be expected to be similar to the Agency's, reducing greatly their corroborative value.

Limitations in the Review: Some Panel members expressed concerns with the Agency review of the supporting data as follows:

1) Comment on the Adequacy of the Approach Used by the Agency

The available data for the study included 304 pesticide studies. The EPA chose to focus its analysis on 77 of these studies in which: a) testing in dogs was used to establish the final RfD (116 of 304 studies); and b) where 13-week or interim data were available to compare to the results of the 1-year chronic study (77 of the 116). Of the 77 studies meeting these criteria, 42 provided data for independent 13-week and 1-year studies. The remaining 35 studies provided interim data (e.g. 13-weeks) from animals that were continuing on the 1-year chronic study. The 13-week and chronic results for these latter 35 are therefore correlated statistics. If the final assessment for the 1-year studies were blind to the corresponding interim results and there was no significant measurement error at either time point, the intra-individual correlation in these latter studies should actually improve the precision of the contrast between the 13-week and chronic study results. However, unless explicitly established by the testing protocol, it is more likely that the assessor of the 1-year study had access to the 13-week results. If this is the case, the degree of correspondence between the two sets of observations in the same dogs will be greater than if the comparison were based on independent studies.

The criteria by which studies were selected for analysis was discussed at some length by the Panel. Of particular interest was exclusion from the analysis of dog studies for pesticides where chronic rodent toxicity data were the primary basis for RfD determination. Exclusion of these dog studies could be justified if the question to be addressed is simply whether or not the absence of 1-year dog study data would have made a difference in the RfDs for pesticides included in the analysis. In answering this question, it makes sense to focus the analysis on pesticides where dog study data were critical for RfD derivation. However, the Panel thought that a different question is also germane — whether a chronic RfD derived from dog studies can be adequately predicted based on a 13-week study alone (i.e., without also conducting a 1-year study). This question relates to the issue of whether the contribution of dog studies to overall RfD development can be adequately achieved by requiring a study of only 13-weeks duration. For this question, all studies offering toxicity data from both 13-weeks and 1-year have potential value, and much of this information is lost using the Agency study selection criteria.

The exclusion of these studies, plus the exclusions made because comparisons of dog studies of different durations were not available (or were unreliable) reduced the original respectably-sized database of 304 pesticides to a less robust one of 77. Inclusion of additional studies would not only allow more comparisons of NOAELs/LOAELs from the different study durations, but also more test cases to determine whether new effects observed in a 1-year study might lead to application of a different uncertainty factor. The title of the analysis produced by the Agency, "A Comparison of the Results of Studies on Pesticides from 1- or 2-year Dog Studies with Dog Studies of Shorter Duration," suggests that the question is not limited to comparison of 13-week and 1-year studies. Studies shorter than 13-weeks could be included in the analysis. Some members of the Panel considered it appropriate to include data from studies of less than 13-weeks as surrogates for 13-weeks, if the studies are long enough to elicit a response and that response was also observed in the 1-year study. Since there were only 2 chemicals of less than 4 weeks dosing duration (one of 3-days and one of 12-days) out of the 35 in this group and since both elicited responses similar to 1-year, their data could also be used, depending upon how the question being addressed by the analysis is defined.

2) Comparisons Made Regarding the Results of Dog Studies of Different Durations

A full comparison of the results from 13-week dog studies with the results from studies of 1-year or longer duration was possible for only 42 of the 77 pesticides analyzed. Analysis of the remaining 35 pesticides relied on comparing the chronic studies with studies of just 4 weeks duration or (more commonly) on intra-study comparisons of interim and chronic results of blood chemistry and hematology observations without the possibility of comparing histopathology.

There are two important differences between the group of 42 pesticides and the group of 35: (a) histopathological data exists for both the 13-week and the 1-year or longer studies for the 42 pesticides but not for the shorter term or interim data used for

the 35 pesticides and (b) there is a precise correspondence of the doses between the comparisons made for the group of 35 pesticides that is not observed among the 42 pesticides. Thus, the full comparison of all 77 pesticides contains a bias introduced by the inclusion of the 35 pesticides for which no histopathological data are available, although some Panel members did not think this was a serious bias if the same blood chemistry and hematology observations were used for determining both the interim and 1-year or longer RfDs.

The differences between the groups of 42 and 35 pesticides suggest that their data should not be pooled so that the bias can be avoided. In addition, more analysis of the groups of 42 and 35 pesticides, and their differences, is necessary. When the groups are separated, the chronic studies seem to provide no additional toxicological data for the determination of the RfD for the group of 35 pesticides. However, the chronic studies do provide additional toxicological data relevant to the RfD for 19 pesticides from the group of 42. Of the 19, there are only 3 pesticides where the chronic dog study potentially is more appropriate than the 13-week study for the selection of NOAELs and LOAELs. If one accepts the various arguments put forward by the Agency regarding the dose selection and spacing, inter-experimental variability, and other limitations relevant to 16 of these 19 pesticides, then there is reasonable concordance in the results from the different study durations.

The Panel provided the following recommendations for additional analysis:

1) The comparative analysis of the significant (i.e. greater than 1.5X) differences in NOAEL and LOAEL levels established from 13-week and 1-year dog studies is a retrospective analysis of a database compiled from over 20 years of pesticide exposure testing. As is the case in any comparative retrospective study (e.g. case-control), inferences drawn from the analysis assume that all factors that bear on the observations are either random with respect to the outcome or can be controlled through statistical adjustment. The decision by the Agency to restrict its analysis to only pesticides for which the dog data were used to set the RfD requires the assumption that the historical decision to use rodent data to set the RfD was independent of the results for the tests conducted in dogs. This is a strong assumption, though not necessarily an unreasonable one. It is an assumption that the Panel agrees should be tested. The Panel recommends that the Agency extend its comparison of NOAEL and LOAEL values for 13-weeks and 1-year dog studies to include a random sample of those pesticides for which dog studies are available, but the RfD was based on the rodent study data. The purpose behind this additional work would be to test the null hypothesis that the level of agreement in 13-week and 1-year studies results for NOAEL and LOAEL in this previously omitted set of studies is comparable to that established in the 77 studies that are reviewed in the current paper. The Panel suggests that this sample-based investigation be restricted to those pesticides for which tests were performed under GLP standards. The required sample size can be computed using standard sample size calculation programs. The sample size calculation will require the Agency to set an objective criterion (effect size) for determining if the relationship between 13-week and 1-year NOAEL and LOAEL values

for pesticides with RfDs derived from rodent studies is equivalent to the relationship that has already been observed and reported for pesticides with RfDs based on the dog studies.

2) Currently, 13-week studies may not have been reviewed to the same level of rigor as for the 1-year dog studies. Without the knowledge and support from the 1-year study, early response seen in a 13-week study (e.g., minor changes in liver enzyme levels) may be recognized as indicators of exposure rather than adverse endpoints suitable for risk assessment. Thus, without the 1-year study, the NOAEL and LOAEL from a 13-week study may be higher than as presented in the Agency position paper. A more realistic data comparison between the 13-week and 1-year dog studies is to review the 13-week studies independent of the 1-year chronic studies (i.e., as if they do not exist) for establishing an RfD, and contrast this evaluation with the information from 1-year studies. By conducting this exercise, the Agency could also begin to formulate a set of criteria for evaluating the short-term studies (e.g., 13-week study) for establishing the NOAEL/LOAEL.

3) The Agency analysis might benefit from analyzing the major classes of pesticides separately, i.e. herbicides, insecticides, fungicides and others, as was done in the German review, just to make sure that one of these classes is not an outlier. This can be determined by searching the tables, but a simple summary table using the number of chemicals by class would be informative.

4) The following background information, if included in the Agency position paper, can provide a more thorough understanding and perspective for the reader.

- A description of the types of dog data that are submitted to the Agency, e.g. dose selection criteria, number of dose groups, number of animals per dose group, major toxicity endpoints evaluated in a study.

- A description of the nature of the “peer review” conducted for studies submitted to the Agency.

- A definition of an “adverse” effect and its distinction from treatment-related effects that are not adverse. Are there some adverse effects that are of greater significance than others? If so, provide some examples. How do such adverse effects impact upon the determination of an RfD?

- A description of how the RfD is calculated and used. Specifically, in describing the approach to calculating the RfD, include the concept of using "the most sensitive endpoint in the most sensitive species” and “uncertainty factors.” A brief explanation of the relationship between RfD and the often used expression "Margin of Exposure" (MOE) could provide perspectives on how the RfD is used in pesticide risk assessment and regulation.

5) To avoid the apparent discordance between the title of the Agency's position paper, the approach to data selection, and the Agency's conclusion from the analysis, it was suggested that the Agency consider revising the position paper title to better reflect the apparent purpose of the analysis as presented. Based on the selected number of datasets included in the analysis, one suggested title is "A Comparison of the Results from 1- or 2-Year Dog Studies with Dog Studies of Shorter Duration in Cases where they have been used for the Determination of an RfD."

Issue 2: Examples where NOAELs or LOAELs were found to be lower in chronic dog studies than in 13-week dog studies

Nineteen examples (Table A3, Appendix on Agency's position paper) were initially identified where NOAELs or LOAELs were found to be lower ($\geq$ 1.5X) in chronic dog studies than in 13-week studies. Further analyses showed that for 11 of these examples, the observed differences seem to be associated with differences in dose selection or inter-experimental variability (interim data from 1-year studies indicated the same NOAELs/LOAELs could be identified at 13-weeks). For the remaining 8 pesticides (Table 1, main text), there are rat studies that would provide comparable NOAELs/LOAELs to the chronic dog study (2 pesticides) and, for 3 pesticides, data were insufficient for an in-depth comparison of the results of 13-week and chronic dog studies or the data were inconsistent with current biological understanding. Finally, three pesticides were identified where results of the chronic dog study seem to be more appropriate for selection of NOAELs/LOAELs that would be used for derivation of a chronic RfD.

Question 2

Please comment on the clarity and soundness of the evaluations presented on the 19 pesticides where NOAELs or LOAELs appeared to differ between the 13-week and chronic studies. Specifically, do these cases detract from the overall conclusions regarding the value of 1-year dog studies in RfD selection?

Response

The Agency presented clearly the analysis of data for the 19 chemicals, where NOAELs or LOAELs were lower in the chronic dog studies than in the 13-week dog studies. The Panel agreed that the cases where NOAELs or LOAELs appeared to differ between the 13-week dog studies and chronic dog studies detract from the overall conclusions that a 13-week study is sufficient to assess the toxicity of pesticides in dogs for determining an RfD; in addition, these cases serve to identify weaknesses in the existing study designs. However, opinions differed regarding the practical significance of a small difference in the NOAEL relative to the 100-fold default uncertainty factor

commonly used in calculating the RfD.

The more detailed comparison and analysis presented for the 19 chemicals identifies several common issues in data evaluation for hazard identification as well as dose-response assessment. Some of these are: data interpretation for NOAEL and LOAEL determination, uneven quality of studies within a database for the NOAEL and LOAEL comparison, different levels of detail in toxicological examinations among these studies, and experimental variability that is further compounded by the relatively small sample size in dog studies. New and more severe or adverse endpoints also can arise in longer-term studies as critical effects at the LOAEL. For example, in the case of cypermethrin, death is one of the observed endpoints at the 1-year study LOAEL. This death occurred at a lower dose than the NOAEL from the 13-week study in which no deaths were observed (page 20 of the Agency position paper "A Comparison of Results from 1- or 2-year Dog Studies on Pesticides with Dog Studies of Shorter Duration," dated 4/7/2005). Even in cases where new endpoints from the 1-year study do not alter the NOAEL determination, such studies can add greater weight to the toxicological evidence that may otherwise be judged as of equivocal adversity based only on the 13-week study. Another issue is the dose spacing and selection. Within the current battery of toxicity tests, the 13-week studies often serve to guide the dose spacing and selection for the 12-month studies. Thus, as can be expected, dose selection contributed to a majority of the NOAEL and LOAEL differences between the 13-week and 1-year dog studies presented in the Agency analysis. More importantly, within the NOAEL/LOAEL approach to dose-response assessment, dose selection is a significant factor that will directly impact the ultimate determination of NOAEL for the RfD calculation. These issues should be taken into consideration in drawing conclusions from the comparative analysis of the NOAEL and LOAEL. Moreover, these considerations will greatly facilitate the setting of the study protocol for 13-week studies and deriving guidance for their evaluation should the Agency decide to eliminate the 1-year study.

The Panel discussed the practical impact of the 1-year dog study on the overall RfD determination. One debate centered on the quantitative significance of a different NOAEL that could result from not having the 1-year studies. On the one hand, a small difference in the NOAEL can be relatively minor compared to the 100-fold uncertainty factor commonly applied to the RfD derivation. On the other hand, since the current default practice is to consistently apply the 100-fold uncertainty factor for inter-species and inter-individual variations in calculating the RfD from all critical NOAELs, a given magnitude of difference in the NOAEL would proportionally affect the final regulatory levels and can be significant to the regulated communities. Another Panel debate was on the significance of missing new or more adverse endpoints that are elicited beyond 13 weeks of exposure but at the same dose level as the 13-week LOAEL. On the one hand, as long as the NOAEL remains the same, identifying more adverse responses in longer studies would not have practical impact on the RfD determination. However, the additional information could provide the certainty and support for effects of equivocal adversity after 13-weeks of dosing. The new and more adverse endpoints from a chronic study would also provide a more accurate picture of the potential risk of long-term

exposure when the RfD is exceeded. The Panel encouraged the Agency to include these issues in their future document.

Issue 3: Recommended durations for dog studies conducted on pharmaceutical agents versus dog studies conducted on pesticides.

An International Conference on Harmonization (ICH) workgroup recommended that, in the case of pharmaceutical agents, the animal toxicity database should include a dog study of at least 9 months duration (DeGeorge *et al.*, 1999). This recommendation appears to have been based primarily on evidence that additional toxicities were seen from 9-12 months that were not evident at 6-months. EPA understands that such studies are often conducted with dose levels in the range of dosages anticipated for humans. Therefore, such evidence could lead to an adjustment in the pharmacologically active doses that could be used clinically or the additional toxicities could be used in the monitoring of clinical parameters. However, it appears that only in a few cases did the additional toxicities seen in 9-12-month studies lead to a revision in the margin of safety (*i.e.*, the ratio of no observed adverse effect level to the anticipated human exposure, in this case exposure to a pharmacologically active dose); it is unclear to what extent margin of safety estimates for pharmaceuticals, in general, would be affected.

EPA's purpose for requiring dog studies conducted with pesticides is to identify a no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL, or the lowest dose that produces some biologically significant evidence of toxicity). The effects at the LOAEL are used to characterize the toxicity that may be expected to occur if exposure to a pesticide exceeds an RfD. The NOAEL is used to derive an RfD, which represents a dose that is unlikely to produce adverse health effects in humans exposed to environmental residues of a pesticide at or below the RfD. In contrast, dog studies performed with pharmaceuticals are designed to ascertain whether adverse effects may occur in humans administered a pharmaceutical chemical at pharmacologic doses and to determine margins of safety.

The analysis of 13-week and 1- or 2-year dog studies conducted on 172 pesticides by Spielmann and Gerbracht (2001) and the results of the analysis of 77 studies on pesticides by the Office of Pesticide Programs has provided evidence that the NOAELs and LOAELs observed in 13-week and 1- or 2-year dog studies generally do not differ. Further, Spielmann and Gerbracht (2001) found that for only 7 of 141 pesticides were significant new effects found in 1- or 2-year dog studies that were not seen in 13-week dog studies or chronic studies with rats. Based on the results of these analyses of dog studies conducted with pesticides, EPA has concluded that a dog study of 13-weeks duration provides sufficient data for an evaluation of potential chronic toxicity in this species along with other routinely required toxicity studies. (Note that a chronic dog study is only one of several studies that may be selected for the derivation of a chronic RfD; other possibilities include a 2-year rat study, a 2-generation rat reproduction study, and an 18-month mouse study).

Question 3A

Given the somewhat different objectives of dog studies conducted on pharmaceutical agents and on pesticides please comment on the extent to which the recommendations of the International Conference on Harmonization workgroup may be relevant to the use of dog studies in risk assessments with pesticides and whether the results of studies performed specifically on pesticides support EPA's conclusion that a dog study of 13 weeks duration along with rodent chronic data is adequate for providing an assessment of chronic toxicity for purposes of deriving chronic RfDs.

Response

While a database for comparison of dose duration effects could contain information derived from testing of pesticides and pharmaceuticals, the objectives of such testing differ for the two classes of products, and this can be considered to limit the utility of merging the data or conclusions. Application of toxicity findings in test species differs between pharmaceuticals and environmental agents on several counts, and as such the conclusions for pharmaceuticals are not directly relevant to findings influencing RfDs. Studies of pesticides focus on testing for safety related to low level environmental exposures, while pharmaceutical testing focuses on effects at or above the pharmacologically active range. The EPA's application of the toxicity findings is more focused on determination of "virtually safe" exposures (doses) as a derivative of toxicity observations. Many-fold margins are applied to findings and the "safe" exposure is derived from the most sensitive endpoint from any study in any species. The purpose of pharmaceutical toxicology studies is not to determine virtually safe doses, but primarily to define the toxicity profile to anticipate what toxicities may occur under conditions of human testing, employing doses that are high relative to those tested for pesticides in the animals. Clinical trials are routinely conducted above the NOAEL or adverse effect level in either or both test species. Thus, findings that occur at exposures higher than the LOAEL are particularly significant in evaluating potential risks to subjects in these studies. Newly observed findings in longer term studies, even when not establishing new safety NOAELs, can contribute to significant, relevant safety concerns for subjects in the clinical studies. These findings above the LOAEL are less of a consideration in defining the RfDs for pesticides.

A further difference between pharmaceuticals and pesticides is use of data from dog studies in the process of safety evaluation. Dog studies are conducted to support clinical trials in drug development. Clinical trial safety data will usually supersede the preclinical data (including dog toxicology information) prior to marketing the pharmaceutical. For pesticides, however, the 13-week and 1-year dog studies are a part of the toxicological program that will be used directly in the final human health risk assessment and to set regulatory exposure standards to prevent any adverse public health effects that will be monitored in post-marketing surveillance programs.

Despite these differences in application of the dog data, the Panel agreed that the data from pharmaceutical experience were informative. Some of the Panel members noted that the retrospective analysis of the pharmaceutical data supported the reduction of the length of dog studies for pesticides. However, other Panel members, given the totality of the data available, did not revise their view that the duration of the dog study could not be reduced in accordance with the EPA proposal.

In the view of several Panel members, the safety derived from an RfD calculation, because of the 100 plus fold uncertainty factors applied was robust enough that it mitigated the small changes in NOAEL/LOAEL observed in the overall dataset, rendering them less meaningful. Given these observations in conjunction with the totality of the data presented, the Panel discussed two potential paths forward. One path forward would be to retain the one-year dog study requirement until limitations in the Agency analysis have been addressed. If the results of the analysis continue to indicate little added value from the one-year dog studies, the Agency could move toward eliminating them on a stronger basis. Another path forward would be to move immediately toward eliminating the one-year dog study requirement, but employ an uncertainty factor until such time as the limitations in the analysis have been addressed. At that point, if there is confidence that data from chronic rodent and 13-week dog studies are sufficient for deriving RfDs, the uncertainty factor could be removed.

One conclusion regarding the pharmaceutical dataset is that its prominence and discussion in the report were excessive in relation to discussions of the pesticide dataset. Regardless of these divergent views on the application and extent of discussion of the pharmaceutical data and its relevance to pesticides, an important observation is that the pharmaceutical data underwent a review process under the auspices of The International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH). This process was critical in standardizing testing recommendations for pharmaceuticals and it would seem valuable that a similar standardized approach be sought for pesticides through international discussions.

Question 3B

> EPA's analysis showed that there was no additional qualitative hazard information provided by a 1-year dog study which would raise significant concerns that would lead to the application of uncertainty factors in addition to the standard factors when deriving an RfD. Please comment on the soundness of this conclusion.

Response

Based on the data provided, several of the Panel members concluded that the EPA analysis could be viewed as defensible. However, most of the Panel, including several who considered the statement defensible on the face value of the data analyzed, voiced

concern that the data used were not adequately inclusive. A substantial portion of the dog data was excluded because the RfD was based on rodent data, rather than dog findings. Most of the Panel agreed these additional data need to be analyzed before the conclusion to eliminate the longer-term dog study could be fully supported. Some Panel members indicated that they could not comment on the soundness of the conclusion without more details on the type(s) of additional qualitative hazard information provided by the 1-year dog study that might lead to use of an additional uncertainty factor in deriving the RfD. As this can be a subjective decision, some additional discussion of the basis for this determination is needed to assess the soundness of the conclusion.

Some Panel members stated that given the inadequacy of the analysis in relation to this issue, they could not make a recommendation on whether or how large an uncertainty factor would be appropriate to apply to a shorter duration study. In the event that the Agency eliminates the 1-year study from data requirements, some recommended that the use of an additional uncertainty factor be retained as one possible tool for establishing the RfD based on a 13-week dog study if there are indications that the dog may be the more sensitive test species and that the 13-week study cannot sufficiently address the toxicity evaluation that could be anticipated from the 1-year study.

As an illustration of the concern the following is cited. The determination of NOAEL and LOAEL are important for dose-response assessment. Beyond this, the toxicity endpoints and their severity or adversity also should/do contribute to both the hazard identification and the dose-response. In the case of cypermethrin, the endpoint from the 13-week study was tremors, but the endpoint from the 1-year study included death not observed in the shorter term study. Although the NOAEL and LOAEL are the same for both endpoints, the adversity of death as an endpoint should inform and impact the risk assessment. In this case, without the 1-year study, the severity of the endpoint at the LOAEL would be missed.

Issue 4: Refinement of the 13-week dog study

The dog, as a second non-rodent species, is well accepted and established as an important regulatory data requirement.

Question 4

> If the 13-week dog study is considered adequate for hazard identification, how could the best use be made of this 13-week study to characterize potential human health effects (e.g., increase the number of animals evaluated, measuring additional parameters such as blood pressure and ECG, obtaining toxicokinetic data)?

Response

The Panel discussed whether the intent of the question was to focus specifically

on hazard identification, or if suggestions to improve the dose-response assessment value of 13-week studies were also relevant. After querying Agency scientists, the Panel directed their comments to both hazard identification and dose-response assessment.

The existing standardized protocol for the 13-week dog study is not likely to be optimal for determining NOAEL and LOAEL for a given pesticide under consideration. The optimal design to detect the NOAEL and LOAEL for any single pesticide will depend on the underlying (and unknown) form of the true dose/response curve and the inter-individual variability in the effects of interest. The designer of the study can control the statistical power to detect effects (or absence) by increasing the number of doses, optimally spacing the dose levels and increasing the number of subjects tested at each dose level. To investigate how potential changes to the existing 13-week protocol for dog studies might generally improve the power to detect the true NOAEL and LOAEL, existing data from archived studies can be used to estimate the inter-individual variability of responses of interest. Using these estimates from the historical data and the established NOAEL/LOAEL values, standard programs for power analysis could be used to simulate the effect of increasing sample size per dose, numbers of doses and spacing of doses on the power to detect true NOAEL and LOAEL values.

It was generally agreed that refinements of the 13-week dog study would add confidence and decrease uncertainty in the calculation of RfDs compared to the present study protocols. Several refinements were recommended by the Panel.

1) Study design could be optimized by the use of all available data prior to the conduct of the dog studies. These data may include, but are not limited to, structure-activity relationships, physiologically-based pharmacokinetic modeling, etc.

2) Increasing the number of dose groups treated would result in more accurate calculation of RfDs, NOAELs, and LOAELs. It was pointed out that NOAELs must be based on actual doses used in a study, and having a small number of dose groups limits the datasets that can be used to set NOAELs. Present guidelines use three treatment groups, and the recommendation was to consider increasing this, perhaps to four treatment groups. While there was discussion of increasing the number of animals per dose group, it was pointed out that the dog studies are generally not analyzed statistically due to the limited number of animals per group. One Panel member commented that increasing the number per group from four to six or eight would not increase the statistical power to a convincing degree, and increasing the number to do so is impractical. It was noted that the likelihood of observing an adverse outcome was a function of the number of dose groups, the number of animals per dose group, and the spacing of the dose groups. Increasing the number of dose groups would contribute to the other two variables. In Table A3 of the Agency's document "A Comparison of Results from 1- or 2-year Dog Studies on Pesticides with Dog Studies of Shorter Duration," dated

4/7/2005, 11 pesticides were judged to be associated with differences in dose selection and dose spacing. Increasing these two variables could increase the accuracy of calculations of NOAEL/LOAEL for these chemicals. This was also favored as a way to define more accurately the dose-response curve if that would help reduce uncertainty. It was also recommended that a summary of the pertinent current study guidelines be included in the Agency position document as an appendix.

3) Comparative toxicokinetics was widely accepted as providing additional data that could be used to compare the responses between rats and dogs. While extensive studies on additional animals using radiolabeled compounds was not favored, following unlabeled compound in blood, urine and feces during the course of the 13-week study would be practical and add to the strength of the study, especially when the rat and dog results varied widely. Kinetics of absorption and elimination, and bioavailability estimates would be particularly valuable information to acquire. However, this most probably will require an independent study.

4) In vitro metabolism studies could provide important information about similarities and differences in the biotransformation pathways between rats, dogs, and humans. The relevant value of the rodent and dog studies to human health assessment could be better assessed with such metabolism data.

5) There was a suggestion that, even if the 1 year study requirement for pesticide toxicity testing was eliminated, a chronic study could still be required if the shorter (13 week study) resulted in certain "triggers." These triggers may be events such as data from the dog study indicating that the dog is a much more sensitive species than the rat, data from outside sources that indicate that early changes to chemical exposure are not representative of toxicity following chronic exposure, etc.

Additional General Comments from the Panel

In addition to providing comments to the four questions posed by the Agency, the Panel proposed that the Agency consider the following:

The Panel cautioned that conclusions based on analysis of the existing database may not be valid for new chemicals undergoing review or in development, especially if they have different modes of action from those previously evaluated.

The Panel suggested two interrelated approaches to assess the impact and role of dog studies in the RfD determination. First, instead of the 1.5-fold impact on the RfD used by the Agency as the criterion for data comparison between the 13-week and 1-year dog studies, the Agency may consider assessing the impact of requiring only a 13-week

study against a pre-defined magnitude of deviation from the current RfD as the worst case scenario. Second, assess whether any dog study is needed to establish the RfD. To answer this question, the Agency may want to re-evaluate their dataset, comparing the RfD that would be calculated from the appropriate rodent study to the RfD derived from the dog study and determine the magnitude of their differences. If the difference is never greater than "x", then it may be possible to use only rat data with an additional uncertainty factor of "x". If the RfD with this additional uncertainty factor is acceptable (assures safety) for a given pesticide there may not be a need for dog data, unless there is a question about target organ toxicity (qualitative).

In assessing the usefulness of the 1-year dog study, the Agency should also consider the potential consequence of seeking to rely on a single test with a study length that is short in comparison with the normal lifespan of the animal. The specific concern is the possibility of not attaining the purported advantage of using fewer animals. One scenario is the potential for poorly defined NOAEL due to the sub-optimal spacing and range of doses that can result from the absence of a range finding study. In this case, instead of using a single study for both hazard identification and dose-response assessment, a repeated study may become desirable merely for re-defining the NOAEL. The shorter duration of the study is likely to make the decision to repeat a study more desirable and attainable than would a long-term study. However, a repeated study practically nullifies the purported advantage of reducing the use of animals in toxicity testing. Another scenario is the need to more carefully consider the design and conduct of the 13-week study to assure the detection of adverse effects when a 1-year study is no longer available to confirm and support the findings from a single 13-week study. One way to strengthen the 13-week study is through increasing the number of dose groups over the current protocol of employing 3 treatment groups. However, increasing dose groups would also mean less reduction of use of animals from the current testing requirements. In order to retain the purported desire to reduce the use of animals, a third scenario may be to require the chemical sponsors to abide by the NOAEL established from the single dog study that meets the prescribed study protocol without the recourse of repeating the study, even if the resulting NOAEL is lower than desirable.