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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

December 22, 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ***MALATHION*: - RE-EVALUATION** Report of the Hazard Identification Assessment Review Committee.

FROM: Jess Rowland, Executive Secretary 12/22/98
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Chairman, 12/22/98
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Diana Locke, Risk Assessor
Reregistration Branch II
Health Effects Division (7509C)

PC Code: 057701

On November 6, 1997, the Health Effects Division's Hazard Identification Review Committee evaluated the toxicology data base, selected doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure risk assessments, and addressed the sensitivity of infants and children from exposure to malathion as required by the Food Quality Protection Act (FQPA) of 1996. The HIARC's conclusions were presented in the committee report issued on December 17, 1997 (*Memorandum:* J. Rowland to A. Nielsen, HED Document No. 012440)

Following that meeting, the Agency pursued the external peer review mechanism to address a number of issues raised by Dr. Brian Dementi, the malathion, toxicologist following the November 6, 1997 HIARC meeting. This peer review was conducted by soliciting comments from three experts in toxicology chosen by the Agency. The external peer review panel submitted their responses to the Agency in May, 1998. On August 18, 20 and 27, 1998, the HIARC evaluated the comments and responses provided by the external peer review panel.

These responses, the HIARC's evaluation of the panel's responses and the HIARC's conclusions are presented in this report.

Committee Members in Attendance

Members in attendance were:

William Burnam
Robert Fricke
Karen Hamernik
Susan Makris
Melba Morrow
Kathleen Raffaele
John Redden
Jess Rowland (Executive Secretary)
Clark Swentzel (Chairman)

Data was presented by Brian Dementi of Toxicology Branch 1.

HED staff also in attendance at this meeting were:
E. Budd
S. Dapson
C. Jarvis
M. Lamont
A. Protzel
B. Tarplee
P. Wagner.

Report Preparation: _____

Jess Rowland
Executive Secretary

A. INTRODUCTION

On November 6, 1997, the Health Effects Division's Hazard Identification Review committee evaluated the toxicology data base to select the doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure risk assessments, and addressed the enhanced sensitivity of infants and children from exposure to malathion as required by the Food Quality Protection Act (FQPA) of 1996. The HIARC's conclusions were presented in the committee report issued on December 17, 1997 (*Memorandum:* J. Rowland to A. Nielsen, HED Document No. 012440)

Following that meeting, the Agency pursued the external peer review mechanism to address a number of issues raised by Dr. Brian Dementi, the malathion, toxicologist following the November 6, 1997 HIARC meeting. This peer review was conducted by soliciting comments from three experts in toxicology chosen by the Agency. The external peer review panel submitted their responses to the Agency in May, 1998 **(Attachment I)**.

On August 18, 20 and 27, 1998, the HIARC evaluated the comments and responses provided by the external peer review panel which are presented in Appendix I.

B. BACKGROUND

The external peer review panel (referred to henceforth as the Panel) consisted of three experts in toxicology selected by the Agency: Drs Michale Dourson, Rolf Hartung and Walter Decker. On behalf of OPP, Dr. Brian Dementi of Toxicology Branch 2, drafted a set of questions for the Panel under eight major topics. The Panel received all pertinent reference materials, namely the Data Evaluation Records of the toxicology studies, the One-Liner database and Dr. Dementi's memoranda and set of questions. The eight specific topics identified by Dr. Dementi are presented below. The specific questions for these topics are presented in Section III. HIARC's Evaluation of External Peer Review Panel's Response.

I. Hazard Identification/Acute Oral (One-Day)

II. Determination of Susceptibility, Reproductive Toxicity

III. Hazard Identification/Chronic Dietary (RfD)

IV. Subchronic Inhalation Study

V. Acute Neurotoxicity Study (Retinal Rosettes)

VI. Subchronic Neurotoxicity Study

VII. Cholinesterase Inhibition - Enhanced Sensitivity of Females

VIII. Cholinesterase Inhibition - Chronic Dog Study

Prior to the meeting, individual members of the HIARC with expertise in the areas of topics listed above were assigned to review the Panel responses and present their findings to the Committee. Dr. Dementi presented an overview of the Panel comments and guided the Committee through each topic. The Committee evaluated the Panel' responses and the assessments by the individual HIARC member assigned for each topic in conjunction with the malathion toxicology database.

III. HIARC's EVALUATION OF EXTERNAL PEER REVIEW PANEL'S RESPONSES'

Presented below are the questions presented to the Panel for each topic, a synopsis of the Panels responses and the HIARC's conclusions.

I. Hazard Identification/Acute Oral (One-Day)

Question 1): *Do the rabbit developmental toxicity and developmental range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicological consequence in either the maternal or the developing organism?*

Panels Response: The Panel did not think the Agency's acute dietary endpoint of 50 mg/kg was justified based on the rabbit data and thought that an acute oral study measuring cholinesterase would be better.

HIARC's Conclusion: The Committee concluded that based on the combined results of the Range-Finding and Main Rabbit development study, a single oral dose of 50 mg/kg could be estimated to have no toxicological effect (i.e.,NOAEL) and thus is appropriate for acute dietary risk assessment. This dose was selected from a compilation (synthesized) of studies and is considered to be conservative for a single exposure (acute) dietary risk assessment.
The rationale for sustaining 50 mg/kg/day as the NOAEL for acute RfD is as follows: In the Range-Finding study no deaths occurred at 100 mg/kg/day. Death attributable to a single dose (i.e., the period of exposure of concern) occurred only in 1 doe on GD7 at 400 mg/kg/day and in does at 200 mg/kg/day after multiple doses (i.e., gestation days 11 and 17). Clinical signs seen in both studies were not attributable to a single dose. In the Main Study, the LOEL of 50 mg/kg/day was based on decrease in mean body weight gains in does during the dosing period. This decrease in mean body weight gains was not attributable to a single dose but rather to multiple doses. It should be noted no mortalities, clinical signs or decreases in body weight gain were seen when the same dose was tested in the Range-Finding study. Thus, toxicological endpoints (e.g., death, clinical signs, or certain developmental abnormalities) attributable to a single dose were not observed in does at 50 mg/kg/day. Also, this dose was selected after review of the other oral studies (which are suitable for use in this risk assessment) that had much higher NOELs/LOELs such as the acute neurotoxicity study in rats (NOEL=1000 mg/kg/day, LOEL = 2000 mg/k) and the developmental toxicity study in rats (maternal NOEL=400 mg/kg/day, LOEL=800 mg/kg/day, developmental NOEL=>800 mg/kg/day). In particular, the acute neurotoxicity study in rats was not useful since cholinesterase data in this study showed much variation and a poor dose response relationship and thus was not appropriate for a regulatory endpoint.

Question 2): *Do data on maternal body weights and body weight gain now available in App. III of the rabbit development toxicity study, alter the assigned LOEL/NOEL for the study and does it influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxic effect?*

Panels Response: The panel was not influenced by the new data but thought it showed slight toxic effect at 50 mg/kg, but data were not relevant for single exposure at this dose..

HIARC's Conclusion: The HIARC, again based on the weight-of-evidence of the data base (see rationale above for question 1), reaffirmed its original conclusion that 50 mg/kg/day is appropriate for acute dietary risk assessment.

$$\textbf{Acute RfD} = \frac{\text{50 mg/kg/day (NOEL)}}{\text{100 (UF)}} = \textbf{0.5 mg/kg/day}$$

Question 3): *As presented in a published work in the open literature, a single intraperitoneal dose as low as 50 mg/kg/day in the rat reportedly elicited a clear effect on avoidance performance while cholinesterase inhibition (erythrocyte) was observed at 100 mg/kg. Plasma and brain cholinesterase were also inhibited at 150 mg/kg. Cholinesterase inhibition and decrements in behavior were all very significant though transient effects: a) What level of confidence should be accorded this study?; b) What is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg serve as an endpoint for acute dietary (one-day) risk assessment?; c) Is the data available in the developmental toxicity studies sufficiently reliable to discount the 10x safety factor required under FQPA?.*

Panel's Response: One member accorded low level of confidence to the intraperitoneal (i.p) study because i.p cannot be directly compare to relevant real-life exposure scenarios. The second stated that the intraperitoneal route is of questionable surrogacy for realistic environmental exposures. While, the third member reported that the study has the advantage of testing a relevant effect, he also stated that the route of exposure is an issue.

HIARC's Conclusion: The HIARC considered this route to be not appropriate for acute dietary risk assessment.

*II. **Determination of Susceptibility, Reproductive Toxicity***

***Question 1)**: Can the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation reproduction study in the Sprague-Dawley rats be dismissed as ".....not a true indication of increased sensitivity of offspring....." for the reasons stated in the Hazard ID Committee report?.*

Panels Response: Two panel members stated that there is evidence indicating greater sensitivity (with qualifying remarks) while one stated that there is no indication for greater sensitivity

HIARC's Conclusion: In the two-generation reproduction study, for parental systemic toxicity, the LOEL was 7500 ppm (612 mg/kg/day in males and 703 mg/kg/day in females) based on decreased body weights in F_0 generation during gestation and lactation and decreased body weight in F_1 during pre-mating. For parental systemic toxicity, the NOEL was 5000 ppm (394 mg/kg/day in males and 451 mg/kg/day in females).

The HIARC concurred with the NOEL/LOEL established by the reviewer in the Data Evaluation Record and reaffirmed the initial conclusion that the adult body weight gain data are confirmation of parental toxicity although it is recognized that the weight-of-evidence is not strong since there is lack of concordance between generations, and because the dose response is not pronounced. Nevertheless, the body weight decrements in F_0 females during gestation and lactation are valid and related; the weight decrements established in gestation are maintained during lactation, and can be attributed to maternal toxicity rather than to factors related to the pregnancy, such as litter size or weight.

The lack of a "significant" body weight gain difference during lactation is not sufficient evidence to discount the statistically significant decreases in mean body weight that were observed. Although the decreased body weight values of F_1 males, without concurrent body weight gain deficits, are not strong evidence of toxicity since F_1 weanling pups were significantly smaller, it was also noted that the males did not regain any of the weight deficits initiated in early life. If there were a total lack of parental toxicity at the highest dose tested, the body weight gains of the males may have demonstrated some recovery. Also, it was noted that the body weight data of F_1 females also indicate significant body weight decrements on weeks 1, 8, and 11, but not week 4 (other weeks were not reported). Therefore, the overall conclusion of the Committee was that parental toxicity was demonstrated by the body weight decrements observed.

It was also noted that the treatment level at which parental body weight decrements were observed was substantially (10-fold) greater than the treatment levels at which cholinesterase inhibition was seen in the chronic rat study with malathion. Although cholinesterase measurements are not recommended by the guidelines, and were therefore not performed, it is assumed that cholinesterase inhibition was indeed occurring in the parental animals which were maintained on test substance for at least 10 weeks premating and through approximately 8 additional weeks of reproductive life. This assumption is made because of cholinesterase inhibition observed in subchronic (13-weeks of dietary administration) and chronic studies with rats.

For offspring toxicity, the NOEL was 1700 ppm (131/153 mg/kg/day in males and females) and the LOEL was 5000 ppm (394 mg/kg/day in males and 451 mg/kg/day in females) based on decreased F_{1a} and F_{2b} pup body weights during lactation.

At the November 7, 1997 meeting it was determined that even though the offspring NOEL (131/153 mg/kg/day in M/F) was lower than the parental systemic toxicity NOEL (394/451 in M/F), this was not a true indication of increased susceptibility since: (i) pup body weight decrements were primarily seen at postnatal day 21; (ii) they are likely related to higher consumption of treated feed in late lactation; (iii) there is an assumption that malathion was present in the milk; and (iv) the pups were exposed to the compound both via the feed (at a high relative intake level) and the milk during late lactation, and were receiving an exaggerated dose of the test substance.

The, HIARC reaffirmed its previous conclusion that there is no increased susceptibility and that even though "quantitatively" there appears to be increased susceptibility based on the NOELs/LOELs. "Qualitatively" the "apparent" susceptibility is due to the assumed higher consumption of treated feed in late lactation and the assumed presence of malathion in the milk. The presence of the chemical in the milk is a generic assumption made during hazard assessment for all chemicals (unless we have data to show otherwise), and is not unique for malathion.

Under the current HED Standard Operating Procedures, the HIARC is not responsible for determining the retention, reduction or removal of the 10x safety factor. That determination was made by the FQPA Safety Factor Committee on June 15, 1998. The FQPA Safety Factor Committee evaluated the hazard and exposure (dietary, drinking water and residential) data and concluded that the 10x safety factor for the protection of infants and children (as required by FQPA) should be removed due to 1) completeness of the toxicology database; 2) lack of increased susceptibility in developmental and reproductive toxicity studies; and 3) the use of adequate data (actual, surrogate, and/or modeling outputs) to satisfactorily assess dietary exposure and screening level drinking water as well as residential exposure assessment.

Question 2): *In the absence of assessments of cholinesterase inhibition and behavioral effects testing in adult and young animals in reproduction studies, can the data obtained in the FIFRA guideline study be considered adequate to address the question of whether young or mature animals are more sensitive to malathion?.*

Panel's Response: The panel appears to agree in saying no to this question, i.e., data in the 2-generation reproduction study are not adequate to address the question of relative sensitivity of younger versus mature animals.

HIARC's Conclusion: The adequacy of the two-generation reproduction study to assess increased susceptibility is a generic issue, applicable to all chemicals, and not specific to malathion. At present the determination of susceptibility is made not based on the results of one study but rather on a weight-of-evidence basis that includes acute and subchronic neurotoxicity

studies, the prenatal developmental toxicity studies in rats and rabbits, the 2-generation reproduction toxicity study in rats as well as the toxicity profile of the chemical. The HIARC, in previous deliberations, has determined that, based upon the weight of the evidence, a developmental neurotoxicity study (which assesses behavioral effects in the offspring, as well as many other endpoints, and could potentially include cholinesterase inhibition for perinatal animals) would not be required for malathion at this time.

Question 3): *Does this two-generation reproduction study provide the reliable evidence of no increased sensitivity in pups when compared to adults, as required under FQPA, to discount the 10x safety factor imposed by FQPA as additional protection for infants and children?*

Panel's Response: One panel member suggested a 3x safety factor as opposed to 10x, while acknowledging that the 10x may still be useful as a management tool. The other two panel members said no, though, one member argued that the offspring must be shown to be less sensitive.

HIARC's Conclusion: The HIARC determined that the two-generation reproduction study submitted in support of malathion reregistration provided adequate and reliable data regarding reproductive toxicity and offspring effects, according to Agency guideline recommendations (83-4) and Good Laboratory Practices. The hazard and dose-response assessments are considered by the FQPA Safety Factor Committee along with the dietary (food and water) as well as residential exposure assessment during risk characterization in order to arrive at a determination of whether or not to recommend retention of the 10x FQPA Safety Factor. **This determination cannot be made based upon the hazard assessment of a single toxicity study.**

III. Hazard Identification/Chronic Dietary (RfD)

Question 1): *Given the evidence of a post 3 month recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, can 50 ppm be concluded to have been a NOEL for the first three months of testing?*

Question 2): *Alternatively, do these findings suggest flawed cholinesterase methodology, and if so, what corrective measure could be pursued?*

Question 3): *Should 4 mg/kg/day, the NOEL for plasma cholinesterase inhibition in males, be supported as a replacement for human data previously relied upon in establishing the RfD, or should additional testing be required in the rat to identify a NOEL for cholinesterase inhibition, particularly in females?*

Question 4): *Given that an explanation exists for greater sensitivity of humans than rats with respect to cholinesterase inhibition from malathion exposure (i.e., the lack of carboxylesterase in human plasma) should a 10x safety factor applied to the rat data to allow for "uncertainties" in inter-species variability be considered adequate if the rat data is to be used in deriving the RfD?*

Question 5): *Further, given the RfD based on human data (0.023 mg/kg/day) is lower than that derived from the rat data (0.040 mg/kg/day) and that an explanation exists for a greater sensitivity for humans, should the RfD based on human data be retained?*

Question 6): *Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10x safety factor imposed under FQPA for the protection of infants and children?*

Panel's Response: In their responses to these six questions, the panel made several assertions, suggestions and recommendations with regard to: (i) establishing a NOEL for the first three months in the two-year rat study (*Question* # 1); (ii) the adequacy of the cholinesterase methodology (*Question* # 2); (iii) the need for additional testing to identify a NOEL for cholinesterase inhibition (*Question* # 3); (iv) the need for additional uncertainty factors to account for deficiencies (*Question* # 4); and (v) the discounting of the 10x factor (*Question* # 6).

With regard to question #5 whether the human study should be retained for deriving the RfD, two members said yes, the human study should be retained since human is the correct species of concern while the third member said no, *"the rat study appears to be a stronger basis for RfD than human work"* but advocated *"a 3-fold uncertainty factor to account for deficiencies in the database, principally because the critical effect was not monitored in the two-generation reproduction study in a potentially sensitive subgroup (i.e., young rats)".* This member also suggested that should the human study be retained, an additional uncertainty factor of "unspecified magnitude, probably less than 3, be applied" since human females were not tested.

HIARC's Conclusion: The HIARC reaffirmed its decision to derive the chronic RfD based on the NOEL of 4 mg/kg/day established in the combined chronic toxicity /carcinogenicity study in rats and the use of a UF of 100 to account for inter-species extrapolation and intra-species variation. The RfD remains at 0.04 mg/kg/day.

The HIARC concluded that the human study is not appropriate based on the following factors: (i) there is the low confidence in the human study because of possible confounding factors (e.g., smoking), the purity of malathion is unknown, and the raw data is unavailable for proper evaluation (published in 1962 in open literature); (ii) purity of malathion tested in the animal study is known (97.1%); (iii) the NOEL in the two-year rat study is supported by the NOEL of 4 mg/kg/day established in the subchronic neurotoxicity rat study (based on inhibition of cholinesterase activity); and (iv) the animal toxicology data base is complete except for the subchronic feeding study in dogs and an subchronic inhalation toxicity study in rats.

The HIARC also concluded that an no additional uncertainty factors are necessary since: (i) a NOEL (not a LOEL) was used to derive the RfD; (ii) this NOEL is supported by the same NOEL in the subchronic study in the same species (rats) for the same effects (cholinesterase inhibition) indicating no cumulative toxicity response over time; (iii) the RfD of 0.04 mg/kg/day derived using an animal study with a UF of 100 (for inter-species extrapolation and intra-species variation) is comparable to the RfD of 0.02 mg/kg/day that can be derived by the use of the NOEL of 0.23 mg/kg/day from a human study and a UF of 10x for intra-species variation.

IV. Subchronic Inhalation Study

Question 1): *Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngeal degeneration/hyperplasia supportable?*

Panel's Response: One member recommended against the use of additional UF, another, recommended a UF of 10, while the third member did not feel qualified to answer this question.

HIARC's Conclusion: The HIARC concluded that a Margin of Exposure of 1000 is required for Short-, Intermediate- and Long-Term inhalation exposures. The MOE of 1000 includes the conventional 100 and an additional 10 for the use of a LOEL and the severity of the nasal lesions.

This decision was based on the results of a two-week range finding study (MRID No. 44554301) which was not available to the Committee at the November 6, 1997 meeting. In that study, there was a dose-related increase in the lesions of the nasal cavity (hyperplasia and respiratory epithelium) which was similar to the laryngeal and nasal cavity lesions seen in the subchronic study.

Question 2): *A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not establish NOELs for cholinesterase inhibition or histopathology findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to evoke an uncertainty factor for acute risk assessment (i.e., 1-7 days) on the basis of cumulative effects?*

Panel's Response: Conclusions from two members suggests that the cholinesterase inhibition is well characterized and that an extra UF is not warranted. The third member recommended against using this study since such studies (range finding) do not provide reliable information.

HIARC's Conclusion: The HIARC concluded that based on the availability of the new data (the range finding study), a MOE of 1000 is required also for Short-term inhalation risk assessment (previously it was determined that a MOE of 100 is adequate for this exposure period).

Question 3): *Should another study be required to identify the NOEL for the end points in question ?*

Panel's Response: One member would like to identify a NOEL, while the other suggests first using bench mark approach. The third does not want an inhalation study with rats.

HIARC's Conclusion: The HIARC determined that a new inhalation study is required based on the results of the two-week range-finding study (MRID No. 44554301) and the lack of a NOAEL for cholinesterase inhibition in the 90-day study (MRID No. 43266601).

Question 4): *Given the findings of nasal and laryngeal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalation route (e.g., inhalation exposure for first 90 days of a two year study)?*

Panel's Response: One member said yes to requiring this study, another member does not want this study and the third member would like to see mode of action studies to understand nasal injury and questions the utility of the inhalation study.

HIARC's Conclusion: At its meetings held on September 24, October 8 and October 15, 1997, HED's Cancer Assessment Committee (CARC) determined that in order to conduct an accurate assessment on the relevancy of nasal tumors to malathion exposure, the nasal tissues from all animals from all dose groups in the 2-year carcinogenicity study (MRID No. 43942901) should be evaluated/re-evaluated (*Memorandum:* J. Rowland, to M. Ioannou, dated 11/3/97; HED Document No. 012374). **Therefore, the HIARC concluded that the need for a study will be determined after CARC's review and evaluation of the requested histopathological examinations.**

Question 5): *Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10x safety factor imposed under FQPA for the protection of infants and children?*

Panel's Response: The panel agreed that the study does not provide any support for discounting use of the 10x safety factor imposed by FQPA. One member acknowledged that the study does not evaluate young individuals and asserted that the FQPA 10x factor is a risk management tool and including it in the scientific discussion of database sufficiency is not appropriate.

HIARC's Conclusion: This study is not appropriate for FQPA assessment because: (i) the study was conducted in adult animals; (ii) there was no exposure to pregnant animals nor was there pre/post natal exposure; (iii) this study did not evaluate parameters in fetuses or pups; and (iv) is not appropriate for assessment of increased susceptibility under FQPA provisions.. **Therefore, HIARC concluded that discussion about the FQPA Safety Factor is neither applicable nor appropriate for this study. In addition, the FQPA Safety Factor, when required, is not applied to any single toxicity study but rather for dietary and residential exposure risk assessments.**

V. ***Acute Neurotoxicity Study (Retinal Rosettes)***

Question 1): *Should retinal histopathology data be submitted for rats in the intermediate dose groups?*

Panel's Response: Two of the members said yes. The third member suggested that the decision to evaluate lower dose groups be made after re-evaluation of the slides in question.

HIARC's Conclusion: The HIARC noted that this issue of reexamination of the retinal tissue of three rats was addressed by an *ad hoc* subgroup of neurotoxicity experts in HED.

The *ad hoc* group met on November 13, 1997, and after careful evaluation of all available data, concluded that the Agency should not ask for evaluation of the retinal tissue of three rats in the acute and subchronic neurotoxicity studies. This decision was based on the following weight-of-evidence considerations (*Memorandum:* E. Budd to R. Loranger, dated December 3, 1997).

1) The lesion of concern (bilateral retinal rosette) occurred in only one male rat at the high dose in the acute neurotoxicity study.

2) A unilateral retinal rosette was also tentatively observed in one male rat in the control group in the subchronic neurotoxicity study.

3) Dr. Brennecke (HED's pathology consultant) and Dr. Dahlgreen (the study pathologist) both concluded the retinal rosette in the male rat at the high dose was not of toxicological significance and was not due to treatment with malathion

4) The *ad hoc* group also concluded that retinal rosettes in rats are most likely the result of abnormal proliferation and differentiation of developing retinal cells during neonatal life (i.e., during the first approximately 32 days after birth) and ordinarily are not likely to develop in mature animals as a result of treatment with xenobiotics.

5) An available reference (Ophthalmic Pathology of Animals, Saunders and Rubin, 1975), stated that "[retinal] rosettes occur spontaneously in certain strains of inbred rats and in beagle and collie dogs".

Based on this information, the HIARC differed with the Panel's recommendations and reaffirmed the *ad hoc* group's decision on this issue, concluding that no additional histopathological examination is necessary at this time.

Question 2): *Should histopathology slides be submitted for independent examination by the Agency's pathologist (for anatomic features comparisons between control and treatment group lesions) as called for in the Data Evaluation Record (DER) for this study (a relatively simple request)?*

Panel's Response: All three members responded yes.

HIARC's Conclusion: The HIARC again differed with the panel and reaffirmed the decisions made by the *ad hoc* group based on the rationale provided above.

VI. Subchronic Neurotoxicity Study

Question 1 *Given the contrast between the NOEL of 1575 mg/kg/day (HDT) for female rats on neurotoxicity endpoints in this FIFRA Guideline study and that of the LOEL of 38 mg/kg/day (LDT) in the published work on a different set of neurotoxicity parameters, does the published work provide adequate reason or evidence to require a developmental neurotoxicity Guideline study, or another neurotoxicity study that embraces learning/memory, EEG, EMG, and possibly other neurotoxicity parameters not covered in the subchronic neurotoxicity Guideline study?*

Panel's Response: One panel member said yes. One member questioned the acceptability of the published study. The other member did not believe that the published study provided reason to require additional studies.

HIARC's Conclusion: The *ad hoc* group, after careful evaluation of all available data, concluded that the Agency should not ask for additional neurotoxicity studies on malathion at this time. It was recognized, however, that such studies might possibly be requested at some time in the future if there is sufficient justification for doing so. The group also suggested that additional literature searches should be conducted on learning/behavior effects of organophosphates in general, and available information on malathion particularly (*Memorandum:* E. Budd to R. Loranger, dated December 3, 1997).

The HIARC reaffirmed the *ad hoc* group's decision on this issue and concluded that no additional studies are required at this time. The HIARC also noted that lack of studies that evaluate learning and/or memory or behavioral effects under the Subdivision F Guideline requirement is a generic issue applicable to all organophosphates, and not particular to malathion. The HIARC recommended that the issue of requiring such a study should be evaluated in conjunction with discussion on the data requirements for FQPA.

Question 2): *If the neurotoxicity findings in the published study are considered inadequate to trigger the additional Guideline testing, what criteria from published work, short of those upon which regulations could be directly based, might serve in that capacity?. (Note: Moeller and Rider (1962), a journal publication with attendant Guideline deficiencies, has served for decades as the basis for a regulatable end point (RfD) for malathion, while the publication in question here is only being put forth as sufficiently definitive to require a study in the FIFRA Guidelines heretofore not performed).*

Panel's Response: One member deferred this question to Agency experts, the second member did not provide a response, and the third suggested having a neurotoxicologist provide criteria.

HIARC's Conclusion: As discussed above, the HIARC noted that this is a generic issue that needs further discussion by OPP.

VII Cholinesterase Inhibition - Enhanced Sensitivity of Females

Question 1): *Does the malathion data base support a conclusion that females are the more sensitive gender with respect to cholinesterase inhibition by this organophosphate?*

Panel's Response: One member says may be yes, but not in the two-year study used for establishing the RfD. The second member stated that the data are not presented in a proper manner for this assessment. The third member responded that yes, more data is needed to characterize the gender specific disparity.

HIARC's Conclusion: This issue (the possibility of greater sensitivity in one sex) has surfaced several times in the past with respect to setting RfD for other chemicals and, as a general policy, it has previously been decided that an additional uncertainty factor would not ordinarily be applied to the RfD based on possible sex-related differences

In considering sex related sensitivity to malathion, the entire data base should be examined to see if any peculiarities exist that could serve as a basis for claims of sex-linked sensitivity. If peculiarities are present, they should be further examined to determine whether they are consistent in their occurrence; affecting the same endpoint, and affecting females with the same degree of sensitivity across species lines.

The toxicology profile suggests that overall sensitivity to malathion is similar for both sexes and that there is no reason to believe that females are consistently more sensitive than males. In certain studies (e.g., subchronic neurotoxicity study in rats, the subchronic inhalation toxicity study in rats and the 21-day dermal toxicity study), females do indeed appear to be more sensitive than males as there are indications that the difference in cholinesterase inhibition is at least an order of magnitude when males were compared to females However, there is no clear picture on the relative degree of increased sensitivity of females compared to males when observed. When studies in which females appeared to be more sensitive are further examined to see what compartment of cholinesterase is affected, again there is no consistency. In some cases, the red blood cell and plasma activity appears to be indicators of sensitivity and in other cases, the brain cholinesterase activity appear to be more sensitive. Again, this finding is in studies in which females were designated as having lower NOAELs when cholinesterase was the endpoint of concern. In many (but not all) studies, the sex-related difference did not result in different cholinesterase NOELs for males and females, but rather in different degree of cholinesterase inhibition for males and females at a given dose level. The HIARC noted that NOELs, rather than degree of effect at a given dose level, are used to derive the RfD.

Question 2): *What approach might be taken to estimate, from the data currently available, a correction factor to be applied to the NOEL derived from the Moeller and Rider study in male human subjects to afford equivalent protection for women?.*

Panel's Response: The members were split on this issue and did not offer any concrete approach to this.

HIARC's Conclusion: The HIARC concluded that even if the human study (where no females were used) had been chosen as the basis for the RfD, it would not be appropriate to apply additional uncertainty factor to account for the increased sensitivity of females as compared to males. The rationale for this decision was that (i) when sex-related difference in sensitivity was observed, the difference appears to be small and (ii) the NOELs, rather than degree of effect are used to derive the RfD. However, the RfD is based on the chronic rat study, an additional factor based on sex would be of no relevance since the NOEL for plasma cholinesterase inhibition in that study was 50 ppm for both sexes (equivalent to 4 mg/kg/day in males and 5 mg/kg/day in females). (Note: one panel member also pointed out that the "*NOELs for cholinesterase inhibition in both male and female rats are the same in the critical study*").

Question 3): *Should additional testing in animal models be required to further quantitate the gender specific disparity?.*

Panel's Response: One member said no. Another suggested the study be extended to include females. The third member said yes, more data are needed to define gender disparity.

HIARC's Conclusion: It was the consensus of the Committee that additional testing is not necessary because: (i) the human study (with one sex) was not used for establishing the RfD; (ii) the NOELs for cholinesterase inhibition in both males and female rats are the same in the critical (animal) study used to derive the RfD (as duly noted by one of the Panel member); (iii) as discussed above, the "apparent" sex difference in sensitivity is not consistent across studies/species (some studies showed fairly large differences); and (iv) NOELs, rather than degree of effect at a given dose level, are used to derive the RfD.

VIII. Cholinesterase Inhibition - Chronic Dog Study

Question: *Knowing that the chronic dog study has no NOEL for cholinesterase inhibition and was considered unacceptable, should additional work, e.g., subchronic feeding study, be required to characterize cholinesterase inhibition in the dog?*

Panel's Response: Two members emphatically responded that no, another study is not required. The third member said yes, because a NOEL is required to comply with the guidelines.

HIARC's Conclusion: The HIARC concluded that a 90-day study in dogs is required based on the rationale provided below: (i) in 1988, the requirement for a subchronic feeding study in dog (§82-1b) was waived contingent upon performance of a chronic toxicity study in dogs; (ii) in waiving this study, there is enhanced burden for the Registrant to provide an acceptable chronic study which was not achieved by the present study (MRID No.40188501); (iii) there are species-related biochemical similarities (absence of plasma carboxylesterase) to anticipate that the dog would respond similarly to man; (iv) since the chronic study was conducted in 1987,

cholinesterase methodology may be problematic and should be examined for conformity with the most current Agency standards; (v) the contrast between doses inhibiting cholinesterase in man and in rat serves to indicate more definitive testing is required in a third species; and (vi) a subchronic study could possibly address the question of whether the type of dosing (capsule vs. dietary) is critical in the dog.

The HIARC recommended that the Registrant consult the Agency for study design and protocol prior to initiation of this study.

C. CONCLUSIONS

1. **No change in the dose and endpoint selected for deriving the acute RfD** at the November 6, 1997 HIARC meeting.

2. **No change in the dose and endpoint selected for deriving the chronic RfD** at the November 6, 1997 HIARC meeting.

3. **No change in the conclusion that there is no evidence of increased susceptibility** in the prenatal developmental toxicity studies in rats and rabbits following *in utero* exposure or in the pre/post natal two generation reproduction toxicity study in rats.

4. **A MOE of 1000 is required for Short, Intermediate and Long-term occupational/residential inhalation risk assessments** instead of a MOE of 100 for Short-term inhalation exposure risk assessment as recommended at the November 6, 1997 meeting.

5. **No additional retinal histopathological examination is required** from the acute neurotoxicity study.

6. **No additional neurotoxicity studies are required at this time.**

7. **The data does not provide a clear picture of enhanced sensitivity in adult females.**

8. **A 90-day feeding study in dogs is required**

9. **A 90-day inhalation toxicity study in rats is required**

D. MINORITY REPORTS

Sixteen "letters" from Brian Dementi, malathion toxicologist, either to Clark Swentzel, Chairman and /or to Jess Rowland, Executive Secretary of the Hazard Identification Assessment Review Committee are provided as Attachments 2 through 18. Attachment 1 contains the responses of the three external peer reviewers and Attachment 2 is the HIARC's report of December 17, 1997.

E. A summary of the doses, toxicology endpoints selected and Margins of Exposure (MOE) dietary and non-dietary exposure assessments are tabulated below..

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY	UF/ MOE
Acute Dietary	NOEL =50.0	Maternal toxicity	Range-Finding & Main Developmental toxicity studies - rabbits	UF =100
Chronic Dietary	NOEL=4.0	Inhibition of plasma cholinesterase activity	Combined/Chronic Toxicity Carcinogenicity - Rat	UF = 100
Short-Term (Dermal)	NOEL =50.0	Inhibition of plasma, RBC and brain cholinesterase activity	21-Day Dermal - Rabbit	MOE =100
Intermediate-Term (Dermal)	NOEL=50.0	Inhibition of plasma, RBC and brain cholinesterase activity	21-Day Dermal - Rabbit	MOE = 100
Long-Term (Dermal)	NOEL=4.0	Inhibition of plasma cholinesterase activity	Combined/ Chronic Toxicity - Rat	MOE = 100
Short-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBC cholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	MOE = 1000[a]
Intermediate-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBC cholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	MOE = 1000[a]
Long-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBC cholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	MOE = 1000[a]

a = A MOE of 1000 is required (includes the conventional 100 and an additional 10x for the use of a LOEL and the severity of the nasal lesions observed in the two-week range finding study).

NOTE: The Aggregate Risk Index (ARI) should be used since different MOEs are required for dermal (MOE=100) and inhalation (MOE=1000) exposure risk assessments.

E. ATTACHMENTS

Attachment 1	Evaluations by the External Peer Review Members
Attachment 2	Report of the Hazard Identification Assessment Review Committee (12/17/97)
Attachment 3	Letter from Brian Dementi - November 10, 1997
Attachment 4	Letter from Brian Dementi - November 20, 1997
Attachment 5	Letter from Brian Dementi - November 25, 1997
Attachment 6	Letter from Brian Dementi - December 17, 1997
Attachment 7	Letter from Brian Dementi - January 15, 1998
Attachment 8	Letter from Brian Dementi - February 10, 1998
Attachment 9	Letter from Brian Dementi - March 10, 1998
Attachment 10	Letter from Brian Dementi - March 16, 1998
Attachment 11	Letter from Brian Dementi - March 20, 1998
Attachment 12	Letter from Brian Dementi - July 27, 1998
Attachment 13	Letter from Brian Dementi - July 29, 1998
Attachment 14	Letter from Brian Dementi - August 3, 1998
Attachment 15	Letter from Brian Dementi - August 10, 1998
Attachment 16	Letter from Brian Dementi - August 17, 1998
Attachment 17	Letter from Brian Dementi - September 24, 1998
Attachment 18	Letter from Brian Dementi - November 5, 1998

ATTACHMENT 1: Evaluations by the External Peer Review Members

External Peer Reviewer: Walter J. Decker, Ph. D. F.A.A.C.T

DER #1

As a whole, this DER is acceptable. It is of concern, however, that many of the studies their in lack statistical power. This may be the reason why there is a general lack of statistical analyses in the report. The appearance of rarely-found malignant tumors in the nasal turbinates of 2 female rats should be a pointer that more animals should be tested to determine the incidence of said tumors in all dosage groups. These tumors should be further histologically defined.

DER #2

It is not clear why malaxon was studied in this series of assessments of malathion. Granted malaoxon is the "active form" of malathion in the body, but it has been known for years that the biotransformation of malathion to malaxon varies in pharmacokinetics among various species.

Unless there surfaces a rational for testing malaxon in this hazard assessment of malathion, I believe DER#2 is in appropriate in this context.

DER#3

The finding that the increased number of hepatocellular tumors observed in the male mice at 100 ppm as compared to the lower numbers of such tumors observed at 800 ppm is not interpretable, in my opinion. Rather, this part of the study should be repeated. The rest of the study seems to follow the Guidelines well, and appears to be scientifically valid.

DER#4

I agree with the EXECUTIVE SUMMARY that this study is not acceptable and does not satisfy Guideline 83-1 for a chronic toxicity study in dogs because NOELs were not established for cholinesterase activity inhibition for plasma and erythrocytes in either sex. Therefore, it should not be used in construction a hazard assessment of malathion.

DER#5

In general, this study seems to be acceptable and satisfies Guideline 83-4 for a multigenerational reproduction study in rats. There were, however, several study deficiencies pointed out by the reviewers on p.20 of the DER. The significance of these could become a debate of great magnitude, but whether they could adversely affect the study is not clear. An example is that histopathologic examinations were not performed on gross lesions of animals found dead; results of such examination on autolyzed tissue could be intermediate or downright misleading.

DER#6

This DER appears to be acceptable and satisfies Guideline 83-3 for a developmental toxicity study in rats.

DER#7

This DER seems to be acceptable and satisfies Guideline 83-3 for a developmental toxicity study in rabbits.

DER#8

This DER appears to be acceptable and satisfies Guideline 83-3 for an acute delayed neurotoxicity study in hens.

DER#9

On p.2 of the MEMORANDUM, the phrase bilateral retinal rosette is used. This reviewer could not find rosette as a pathological term in any text consulted. What were the structures observed when submitted to histological examination? Lacking an answer to the question, I would recommend that this DER be changed from CORE MINIMUM to UNACCEPTABLE for the section on the eye histopathology. I would agree that this DER be graded core minimum for the reasons cited on p.1.

DER#10

Although this study appears to satisfy the requirement of Guidelines 82-7 for subchronic toxicity determinations, it was correctly pointed out in the Study Classification section that other published data indicate possible evidence of neurotoxicity on parameters not assessed in the 82-7 Guidelines. I recommend a through literature search on theses and that the results be used to construct additional specific neurotoxicity testing to assess for effects on learning, behavior, and EEG and EMG evaluations.

DER#11

On p.2, the term "ratio of rations" should be "ratios for ratios"" At about the time that the Desi paper was published, William Stavinoha (University of Texas Health Science Center, San Antonio) published a paper (not available at the present time to this reviewer, who has recently moved) showing that brain cholinesterase was degraded quite rapidly in brain extraction techniques such as Desi used. This degradation was prevented by microwaving the animal's head before the brain was removed for analyses. Using Stavinoha's technique might provide better answers on ChE inhibition in the brain by malathion. I agree with the Footnote on page 13 that the neurotoxicity and neurobehavioral testing should be greatly expanded in scope, in light of development in these areas during the past decade. The DER should be put "on hold" until these changes are made.

DER#12

I agree that this DER is acceptable and satisfies Guideline 82-2 for 21 day dermal toxicity study in rabbits.

DER#13

It would had been less confounding if the rats were exposed to the malathion aerosols using the nose-only technique, because if rats are exposed in a whole-body chamber, they tend to lick their fur upon which the test substance adhere, thus resulting in both inhalation and oral routes of administration. Otherwise, the study design appear to be adequate. This study seems to be generally acceptable, but does not satisfy all requirements of Guideline 82-4, since, no NOEL was established for plasma and RBC cholinesterase inhibition in female animals or for microscopic lesions of the nasal cavity of the larynx in both sexes.

DER#14

This DER appears to be acceptable and satisfies the FIFRA Test Guideline 82-2 requirements for

bacterial mutagenic data.

DER#15

This DER seems acceptable and satisfies the FIFRA Test Guideline 82-2b requirements for chromosome aberration data.

DER#16

This DER appears to be acceptable and satisfy FIFRA Test Guideline 84-4 requirements for DNA damage/repair (other genotoxicity) data.

DER# 18

The data in this study are considered acceptable, but do not satisfy any guideline requirement. How useful this study will be to the USEPA will largely depend on the resolution of differences of opinion between Dr. Dementi and other members of the Hazard Identification Assessment Review Committee as to whether this human study or more recent animal studies should be used to formulate USEPA policy on malathion toxicity. I recommend that Dr. Dementi's suggestions be actively pursued, that is more studies are needed to fill in the data gaps.

DER#19

This range -finding study is as acceptable as such. It appears that it was designated only as a range finding study, and not to be a definitive treatise on developmental toxicity of malathion to rabbits.

DOCUMENT# 1

This paper seems to eminate from training program for Dr. Feldman under a well known dermatotoxicologist, Dr. Maibach. It will not be useful to the USEPA in preparing a Hazard Assessment document.

DOCUMENT# 2

Studies #1, #2, #3, #4, and #5 are unacceptable and do not satisfy Guideline 84-2 for carcinogenicity studies in mice or rats because of deficiencies in each study. On the other hand, study #6 was well designed, and seems acceptable and satisfies Guideline 830-2 for a carcinogenicity study in ice. However, this bioassay was performed with malaxon, the active metabolite of malathion, which may dilute its impact on the Hazard Identification Assessment of malathion.
The MEMORANDUM, SUBJECT: Peer review of Malathion, dated April 12, 1990 is quite dated because of more recent research on carcinogenicity of malathion. Yet, the studies reviewed therein could certainly be used in the Hazard Assessment report.

Selection of Principal Study

I do not believe that the 2-year chronic feeding study in the rat should be the current selection as the Principal Study because:

1. The route of administration studies is oral, but human would be exposed primarily through inhalation or percutaneously as a result of spraying malathion.
2. Many of the studies there in lack statistical power (this may be why there is a general lack of statistical analyses in the report).

3. The appearance of rarely-encountered malignant tumors in the nasal turbinates demand further testing in a larger group of animals in all dosage groups.

Except as noted in the DER reviews, the data base seems close to complete, although the extent of the literature search is not clear. Recommend that in future reviews, reviewers be provided with a printout of "hits" in the search (at least author, title, journal, year, and abstract if available). In general the critical effects in each DER were appropriately chosen and used in the evaluation (ref.A) except as noted. I am not aware of supporting studies which shore up the use of principal study for the RfD.

Areas I-VIII

I Hazard Identification/Acute Oral (One-Day)

Question 1): No, for reasons cited in the reviews of DER #5 and #9.

Question 2): No, the statistical significance of maternal body weight and body weight gain is not available.

Question 3): a) A low level of confidence should be accorded this study, because:

b) The route of administration (intraperitoneal) cannot be directly compared to dietary administration. Humans are exposed to malathion generally through spraying, where inhalation and percutaneous absorption are important.

c) No, Since younger animals (and presumably younger humans are usually more sensitive, to toxic effects of any chemical, the 10X safety factor should not be discounted.

II Determination of Susceptibility, Reproductive Toxicity

Question 1): No, because some toxic effects have been reported.

Question 2): No, because more behavioral (and learning) testing should be performed, since during the last decade, considerably more oinformation has emerged, information which should be used to upgrade the FIFRA Guidelines.

Quetion 3): No because the evidence seems quite thin.

III Hazard Identification/Chronic Dietary (RfD)

Question 1): No, because a NOEL was not identifies for erythrocyte cholinesterase inhibition among females during the first 3 months of testing.

Question 2): Flawed cholinesterase methodology is possible, but if this were true, all OP pesticides, and not just malathion, would give erroneous results.

Question 3): Additional testing should be required in the male and female rat before any thought is given to replacing the human data relied on to establish a RfD.

Question 4): I believe the 10X safety factor would be reasonable if enhanced rat data (see Question 3)

were used in deriving the RfD.

Question 5): The RfD based on human data should be retained for the present time, because are apparently more sensitive.

Question 6): No, because infants and children are very likely to need additional protection, since, they are, in general, more subject to toxic insult than adults.

IV Subchronic Inhalation Study

Question 1): I do not feel comfortable answering this question, since the derivation of a UF of 3 is not clear to me.

Question 2): Since range-finding studies seldom generate reliable NOELs, this study should not be in deciding whether a UF should be evoked.

Question 3): Yes-common sense dictates that NOELs be identified.

Question 4): Yes, because malathion is usually sprayed, the inhalation route is most important.

Question 5): Not in my opinion! Infants and children are usually more strongly affected by toxic insults than are adults.

V Acute Neurotoxicity Study (Retinal Rosettes)

Question 1: Yes, histopathology data is generally desirable for all dosage groups.

Question 2: Yes, the value of independent histopathologic examination has long been recognized in clinical medicine-why not for research, also?

VI Subchronic Neurotoxicity Study

Question 1): An even more important reason is that during the past decade, many more neurotoxicity detection techniques have emerged and shown to be valuable. These tests were not available when the FIFRA Guideline was promulgated.

Question 2): I suggest that a neurotoxicologist be contacted to advise what criteria from published work might be used.

VII Cholinesterase Inhibition-Enhanced Sensitivity of Females

Question 1): There seems to be enough in the malathion data base to support the conclusion: see my answer to Question 3.

Question 2): I can not answer this question, since it is not in my field of expertise.

Question 3) Yes, more data are needed to more rigorously define the gender-specific disparity.

VIII Cholinesterase Inhibition-Chronic Dog Study

Question): Yes, because a NOEL is required to comply with guidelines.

External Peer Reviewer: Dr. Michale Dourson

The following report places the OPP question in italicized text and the answer in bold text.

Charge to the Reviewers

Do you think the appropriate uncertainty factor was used in the establishment of the RfD?

You are asked to determine if the 2-year chronic feeding study in rat should be the correct selection as the principal study.

You are asked if the data base would be considered to be complete, and the critical effects in each DER were appropriately chosen and used in the evaluation. Are there supporting studies which shore up the use of the principal study for the RfD?

The RfD should be based on the blood cholinesterase endpoint in the 2 year bioassay of Huntingdon Life Sciences (1996) (DER # 1), where the low dietary dose of 50 ppm should be considered as a NOAEL in Fischer 344 males and female rats. The judgment that the low dose is a NOAEL, particularly in females, is based on the following 3 lines of evidence:

i. All measurements of RBC cholinesterase inhibition are statistically unaffected at 50 ppm in both males and females at all times. Nor are the observed RBC cholinesterase inhibition measurements at 50 ppm in either males or females consistently below control values at any time, as might be expected from a real effect, but one that was not otherwise statistically significant (see table of mean cholinesterase values on page 69 of exhibit 7).

a)

ii. The statistically significant effect on RBC cholinesterase inhibition in 100 ppm females at 3 months could be due to

- chance;
- an unusually high control value (the female control value of 1.46 IU/ml is >50% higher than the female value at 6 months and even higher than 50% when compared to the two male control values at 3 and 6 months);
- or---the most likely explanation---the fact that the administered dose was 100 ppm in the diet for the first 3 months; since these animals would be expected to eat more diet on the basis of body weight when they are younger (i.e., =3 months), then when they are older (i.e., 3 to 6 months), their dose at =3 months at 100 ppm would also be expected to be more than the difference between 100 ppm and 50 at 6 months (e.g., 100 ppm x 0.1 food feeding factor ~ 10 mg/kg-day; while 50 ppm x 0.05 food feeding factor ~ 3 mg/kg-day).

1. The 13 week neurotoxicity rat study (DER # 10) tested a similar dose range as the 2 year bioassay and yielded similar results. Specifically, the low dose of 50 ppm diet in the 13 week study was a NOAEL in both male and female Sprague-Dawley rats.

The lack of the monitoring of the critical effect in the developing offspring, and specifically, the lack of such measurement of RBC cholinesterase inhibition in the 2 generation study is a data gap that can best be addressed through the use of a 3-fold uncertainty factor when determining the RfD. The use of this factor is well within EPA's judgment in developing RfDs, when an otherwise complete data base suggested an otherwise unmonitored endpoint that may end up being the critical effect (the effect on which the RfD is based).

I consider the FQPA safety factor as a risk management tool that should be used in the determination of risk management decisions when appropriate. Although this focus of the FQPA safety factor is helpful in assuring that sensitive subgroups---such as children on occasion---will not be missed, it is not needed in a scientific discussion of data base uncertainties and use or non-use of the data base uncertainty factor. Furthermore, the use of an additional uncertainty factor of 3-fold for the data gap specified above, precludes the use of an FQPA safety factor based on scientific reasons.

I Hazard Identification/Acute Oral (One-Day)

Supporting documentation: DER #s 5, 6, 7, 9 and 19; References: A (pp. 3-5), B (pp. 1-4), C, D, E, V and BB (pp. 12-14; 20-22)

Question 1): Do the rabbit developmental toxicity and developmental range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicological consequence in either the maternal or the developing organisms?

The specific question to be addressed with these data are whether or not the NOEL of the likely critical effect after 1 day exposure is determinable. The available data in this review, including the developmental studies in rabbits, do not allow this question to be answered. My expectation is that the critical effect from a single day exposure is cholinesterase inhibition. I suspect that the NOEL for this endpoint after 1 day will be higher than the NOEL of 4 mg/kg-day found in the 2 year rat bioassay. The NOEL might even be as high as 50 mg/kg-day established by the i.p. study mentioned in question 3 below. The developmental studies do not measure cholinesterase inhibition. Do we not have any acute oral studies that test for it?

Question 2): Does data on maternal body weight and body weight gain now available in Appendix III of the rabbit developmental toxicity study alter the assignment of the LOEL/NOEL for the study, and does it influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxicological effect?

The data on maternal body weight gain is potentially not relevant to the discussion of critical effect after acute exposure. Do we not have any acute oral studies that test for cholinesterase inhibition?

Question 3): As presented in a published work in the open literature, single intraperitoneal doses as low as 50 mg/kg in the rat reportedly elicited a clear effect on avoidance performance while cholinesterase inhibition (erythrocyte) was observed at 100 mg/kg. Plasma and brain cholinesterases were also inhibited at 150 mg/kg. Cholinesterase inhibition and decrements in behavior were all very significant though transient effects: a) What level of confidence should be accorded this study?; b) What is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg can serve as the endpoint for acute dietary (one-day) risk assessment?; c) Is the data available in the developmental toxicity studies sufficiently reliable to discount the 10X safety factor required under FQPA?

This study has the advantage of testing for what may likely be the relevant effect. Unfortunately, the route of exposure is an issue. Perhaps I missed this in the package of information for review, but do any of the available kinetic data suggest a likely speed and percent of absorption after oral exposure? If such data were available, then a crude comparison to the i.p. study might be possible, and the results of this latter study might be more relevant to the question that is posed.

I am not satisfied that the potential risk to humans is addressed with the data available in this review package. But more data are probably available to further address this question. A discussion of uncertainty factors for potential data base gaps should be postponed pending the review of these additional data.

II Determination of Susceptibility, Reproductive Toxicity

Supporting documentation: DER: # 5; References: A (pp. 15-16), B (pp. 3-4), F, G and BB (pp. 12-14; 16-17; 20-22)

Question 1) Can the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation reproduction study in the Sprague-Dawley rat be dismissed as "...not a true indication of increased sensitivity of offspring..." for the reasons stated in the Hazard ID Committee report?

The answer here depends in part on how the doses were calculated in the Hazard ID committee report. If the doses for the offspring were simply a reflection of the adult doses (which is what they appear to be), then the apparent greater sensitivity of offspring is offset by their likely higher ingested dose. This apparent sensitivity can thus be discounted based on the reasoning of the committee report. If instead, the offspring dose is calculated from their own food consumption, then the apparent toxicity is real and should not be discounted.

Question 2) In the absence of assessments of cholinesterase inhibition and behavioral effects testing in adult and young animals in reproduction studies, can the data obtained in the FIFRA guideline study be considered adequate to address the question of whether young or mature animals are the more sensitive to malathion?

No, the data on which to make this determination are absent. Perhaps the argument could be made based on analogy to other cholinesterase inhibitors that the young animals in a 2 generation reproductive study are not as sensitive, or are equally sensitive, as adults from a subchronic or 2 year bioassay. But this argument is not made in the materials provided for review.

Question 3) Does this two-generation reproduction study provide the reliable evidence of no increased sensitivity in pups when compared to adults, as required under FQPA, to discount the 10X safety factor imposed by FQPA as additional protection for infants and children?

The absence of determining the critical effect in a potentially sensitive subpopulation necessitates the use of an additional uncertainty factor for data base deficiencies. The value of this factor should be 3-fold, in keeping with previous EPA decisions for the magnitude of such data base deficiencies. A comparison of cholinesterase inhibition between young animals and adults for other similar chemicals may provide sufficient information in order to discount the use of this additional factor.

As mentioned elsewhere in this review, I do not believe that the FQPA safety factor of 10X is appropriate in a discussion of scientific uncertainties used to establish an RfD, although the focus of the FQPA certainly helps scientists assure that sensitive subgroups, including on occasion children, are adequately addressed. As a risk management tool, the FQPA 10X may still be useful for this chemical. However, the use of an additional 3-fold factor for data base deficiencies as noted above precludes the use of an FQPA factor for scientific reasons.

III Hazard Identification/Chronic Dietary (RfD)

Supporting documentation: DERs: #s 1 and 10; References: A (pp. 5-6), B (pp. 4-5), H, I, N (p. 16), R and Y.

Question 1) Given the evidence of a post 3 months recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, can 50 ppm be concluded to have been a NOEL for the first three months of testing?

Yes, the 50 ppm level in the chronic bioassay is a NOEL. The statistically significant effect on RBC cholinesterase inhibition in 100 ppm females at 3 months could be due to

- chance;
- an unusually high control value (the female control value of 1.46 IU/ml is >50% higher than the female value at 6 months and even higher than 50% when compared to the two male control values at 3 and 6 months);

- the fact that the administered dose was 100 ppm in the diet for the first 3 months (the most likely explanation); since these animals would be expected to eat more diet on the basis of body weight when they are younger (i.e., at 3 months), then when they are older (i.e., 6 months), their dose after 3 months at 100 ppm would also be expected to be more than the difference between 100 ppm and 50 at 6 months (e.g., 100 ppm x 0.1 food feeding factor = 10 mg/kg-day; while 50 ppm x 0.05 food feeding factor = 2.5 mg/kg-day).

Question 2) Alternatively, do these findings suggest flawed cholinesterase methodology, and if so, what corrective measure could be pursued?

I have no comment of the cholinesterase methodology. However, the study seems well designed and executed. The change in dose from 100 ppm to 50 ppm based on the interim results at 3 months in females seems reasonable.

Question 3) Should 4 mg/kg/day, the NOEL for plasma cholinesterase inhibition in male, be supported as a replacement for human data previously relied upon in establishing the RfD, or should additional testing be required in the rat to identify a NOEL for cholinesterase inhibition, particularly in females?

The additional testing is not needed. The 50 ppm level is a NOEL in females in this study. 100ppm in females is not a NOEL.

If some scientists are still motivated to pursue whether 50 ppm is or is not a NOEL in females, then a benchmark dose analysis could be done on the existing female responses, using both the 50 and 100 ppm levels (along with higher levels of course), or the results of this 2 year study could be compared with those from the 13 week neurotoxicity rat study (DER # 10) where a similar dose range as the 2 year bioassay was tested and similar results were found. Specifically, the low dose of 50 ppm diet in the 13 week study was a NOAEL in both male and female Sprague-Dawley rats.

Question 4) Given that an explanation exists for a greater sensitivity of humans than rats with respect to cholinesterase inhibition from malathion exposure (i.e. the lack of carboxylesterase in human plasma) should a 10X safety factor applied to the rat data to allow for "uncertainties" in interspecies variability be considered adequate if the rat data is to be used in deriving the RfD?

A crude comparison of NOEL/LOEL between the human and rat studies is possible. The male

human values are 0.23 and 0.34 mg/kg-day; the rat values are 4 and 29 mg/kg-day. Ideally, one would want to match the extent and incidence of cholinesterase inhibition before making judgments about this comparison; indeed a benchmark dose applied to both data sets may allow such comparison. However, the NOELs are only ~20-fold apart; the LOELs are further, but this likely reflects the fact that the rat NOEL and LOEL was further apart than necessary in the rat study. Furthermore, the human data reflect a gavage dosing whereas the rats are continuously fed. Gavage dosing may likely lower the NOEL and LOEL when compared to continuous feeding. Thus, my overall judgment is that a 10-fold uncertainty factor applied to the rat NOEL of 50 ppm is both necessary---and satisfactory. No additional factor is scientifically needed.

Question 5) Further, given that the RfD based on human data (0.023 mg/kg/day) is lower than that derived from the rat data (0.040 mg/kg/day) and that an explanation exists for a greater sensitivity for humans, should the RfD based on human data be retained?

The rat study appears to be a stronger basis of the RfD than the human work. However, I believe that the rat NOEL should be further divided by a 3-fold uncertainty factor to account for deficiencies in the data base, principally because the critical effect was not monitored in the 2 generation reproductive study in a potentially sensitive subgroup (i.e., young rats). The resulting rat-based RfD is lower than the existing value on EPA's IRIS by about 50%. This comparison of rat to human RfD is thus supportive.

One might, however, ask whether a 3-fold data base factor should also be used with the human work. The use of the human data has the obvious advantage of relevance. However, it does not test females, so the NOEL/LOEL range could potentially be lower. The use of the data base factor of 3-fold would also lower this RfD. Alternatively, the human study is by gavage; thus, the NOEL/LOEL range could potentially be higher. These uncertainties might be quantifiable based on an analysis of other data for this chemical, or perhaps related chemicals, but they do operate to cancel each other out. The end result would be that the expected human-based RfD would be somewhat lower than at present, which is in the direction of the rat RfD based on an uncertainty factor of 30 (i.e., 10 for sensitive humans and 3 for data base).

Question 6) Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?

I do not believe that the FQPA safety factor should be considered in a discussion of science behind whether a data base uncertainty factor should or should not be used. Recently I made public comments on the "use of 10x safety factor to address special sensitivity of infants and children to pesticides. I restate some of these comments here as a partial answer to question 6.

One of the intents of the FQPA is to reduce the risk to children from the noncancer effects of

pesticides. This is a noteworthy goal. However, Reference Doses (or RfDs) used by EPA and others as a measure of noncancer risk are thought by many scientist to be doses below the population threshold for adverse effect[1]---and therefore without risk. Obviously, dividing a dose with zero risk by a safety factor of 10-fold still yields a dose with zero risk. The risk is not lessened by the use of a 10-fold safety factor.

I agree with the OPP that the FQPA safety factor of 10-fold is not part of hazard identification, nor dose response assessment. I agree that it is properly called a safety factor, and should not be considered an uncertainty factor, as established by EPA and others.[1] However, the use of the FQPA safety factor of 10-fold does not belong in risk characterization either. Several of the arguments listed by OPP for its use of this safety factor in risk characterization are similar---or identical---for EPA's use of its data base uncertainty factor, used to determine RfDs.[2] Again the FQPA is not an uncertainty factor. It is properly seen as a safety factor and should be used by risk managers, if needed, during the risk management phase of their work.

All of EPA's RfDs for pesticides, and many of those used by other EPA programs, protect sensitive populations---including children.[3] The current scheme of uncertainty factors used to develop these RfDs does not need to be expanded, although it could certainly be further studied and improved.[4] However, many of the older RfDs on EPA's Integrated Risk Information System (or IRIS) do not consider the data base uncertainty factor in developing RfDs. (The use of this factor was standard practice in OPP---but not the rest of the agency until about 1987.) The public health risk to children---highlighted by FQPA---should be carefully considered in reexamining these older RfDs.

IV Subchronic Inhalation Study

Supporting documentation: DERs: #s 1, 2 and 13; References: A (pp. 9-11), B (pp. 5-6), J, N (p. 12) and 0

Question 1) Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngeal degeneration/hyperplasia supportable?

Cholinesterase inhibition does not appear to be statistically significant at the low dose. Thus, it

[1] Dourson, M.L., S.P. Felter, and D. Robinson. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. Regulatory Toxicol. Pharmacol. 24, 108-120.

[2] Dourson, M.L., Knauf, L.A. and J.C. Swartout, 1992. On Reference Dose (RfD) and Its Underlying Toxicity Data Base. Toxicology and Industrial Health, 8(3): 171-189.

[3] EPA. 1998. Integrated Risk Information System (IRIS). Online at www.epa.gov/iris.

[4] Swartout, J.C., Keenan, R.E., Stickney, J.A., Gillis, C.A., Dourson, M.L., Harvey, T., and P.S. Price. (In press with Risk Analysis). A probabilistic framework for the reference dose.

appears that the low dose is a NOEL for this endpoint in both males and females. ((see Table 4 of DER #13). However, the effects in the nasal passage appear to be real and biologically significant (see description and table of incidences on page 14 of DER #13). The extent and severity of these nasal lesions suggest the use of a 10-fold uncertainty factor, rather than a 3-fold factor.

OPP may wish to compare these effects after conducting dosimetry adjustments as per EPA's guidelines for the development of RfCs. This may or may not change the choice of critical effect.

Question 2) A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not identify NOELs for cholinesterase inhibition or histopathology findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to evoke an uncertainty factor for acute risk assessment (i.e. 1-7 days) on the basis of cumulative effects?

A quick comparison of LOELs for plasma or RBC cholinesterase inhibition between the two week and subchronic rat bioassays gives roughly comparable results at doses of 0.45 mg/L (subchronic) and 0.54 mg/L (2 week). The histopathology is also roughly comparable for these two doses between these two studies. Both comparisons should be checked more carefully.

I believe this supports the contention that an extra uncertainty factor for potential cumulative effects is not needed.

Question 3) Should another study be required to identify the NOEL for the end points in question?

I suggest that the incidence of nasal and larynx effects of similar severity (e.g., slight, moderate) be first plotted as a function of exposure (preferably after dosimetric adjustment by way of EPA's RfC methods). This may allow the use of a benchmark concentration approach to the revised incidence data. (The data shown in table 6 of DER #13 would not be useful unaltered as a basis of a benchmark concentration).

A benchmark concentration could also be applied to the cholinesterase effects in this study as well, but as stated in my answer to question 1, I believe that a NOEL for this endpoint is already demonstrated in the study.

Question 4) Given the findings of nasal and laryngeal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalation route (e.g., inhalation exposure for first 90 days of a two year study)?

Perhaps one should first ask for mechanistic studies. It may be more useful to understand the mechanism of nasal injury in eventual extrapolation (by way of a linear or a MOE approach as per draft agency cancer guidelines), rather than spend the time and money on another two year bioassay.

Question 5) Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?

This study does not test the toxicity of malathion in rat pups or young rats, and by analogy in infants and children. See my comment also under question 6 of the previous grouping. The FQPA safety factor is a risk management tool. Its use in a scientific discussion of data base sufficiency is not appropriate. EPA has a data base uncertainty factor for such discussions.

V Acute Neurotoxicity Study (Retinal Rosettes)

Supporting documentation: DER #s 9 and 10; References: L, M and P (pp. 1-2)

Question 1) Should retinal histopathology data be submitted for rats in the intermediate dose group?

It seems reasonable to first ask for the histopathology (as in question 2 below) before requiring additional groups to be analyzed. Retinal histopathology might be a real effect, but only at high doses. Thus, effort to explore the background of this effect might be of academic interest, but have little relevance to the determination of critical effect.

Question 2) Should histopathology slides be submitted for independent examination by the Agency's pathologist (for anatomic features comparison between control and treatment group lesion) as called for in the Data Evaluation Record (DER) for this study (a relatively simple request)?

Yes

VI Subchronic Neurotoxicity Study

Supporting documentation: DER #s 10, 11; References: D, P (pp. 3-4), Q, S, T, U and BB (pp. 12-14; 16-17; 20-22)

Question 1) Given the contrast between the NOEL of 1575 mg/kg/day (HDT) for female rats on neurotoxicity end points in this FIFRA Guideline study and that of the LOEL of 38 mg/kg/day (LDT) in the published work on a different set of neurotoxicity parameters, does the published work provide adequate reason or evidence to require a developmental neurotoxicity Guideline study or another neurotoxicity study that embraces learning/memory, EEG, EMG, and possibly other neurotoxicity parameters not covered in the subchronic neurotoxicity Guideline study?

I do not believe that it does. The LOEL of 38 mg/kg-day for both cholinesterase depression and possible learning effects is not inconsistent with the cholinesterase NOEL of 4 mg/kg-day from the 2 year rat bioassay. A division of this LOEL by a 10-fold uncertainty factor (EPA standard practice with LOELs of this degree of severity) would yield similar values (i.e., 3.8 versus 4 mg/kg-day). The use of a 3-fold uncertainty factor with this LOEL of 38 mg/kg-day would make the cholinesterase inhibition even more significant.

Question 2) If the neurotoxicity findings in the published study are considered inadequate to trigger the additional Guideline testing, what criteria from published work, short of those upon which regulations could be directly based, might serve in that capacity? (Note: Moeller and Rider (1962), a journal publication with attendant Guideline deficiencies, has served for decades as the basis for a regulatable end point (RfD) for malathion, while the publication in question here is only being put forth as sufficiently definitive to require a study in the FIFRA Guidelines heretofore not performed.)

Ultimately, EPA must make the judgment that the neurotoxicity findings in the published study are considered adequate to trigger the additional Guideline study. In this regard, the comments of neurotoxicologists within EPA are valuable. From my general toxicology point of view, one must also judge whether or not the expected NOEL for learning effects will be lower than the NOEL of 4 mg/kg-day for cholinesterase inhibition divided by an additional uncertainty factor of 3-fold for data base deficiencies as I suggest elsewhere in these comments. Therefore, the learning NOEL should be expected to be below ~ 1 mg/kg-day before requiring such a test. My intuition is that a learning effects NOEL would not be lower than 1 mg/kg-day, and thus an additional guideline study is not needed. Again, however, this judgment is best done by experts within EPA.

VII Cholinesterase Inhibition - Enhanced Sensitivity of Females

Supporting documentation: DER #s 1 - 3, 9, 10, 12 and 13; References: W, X, Y, Z and CC

Question 1) Does the malathion data base support a conclusion that females are the more sensitive gender with respect to cholinesterase inhibition by this organophosphate?

The data suggest that this might be the case in the area of dose where cholinesterase inhibition occurs. What is perhaps more important, however, is the value of the NOEL for

cholinesterase inhibition in both sexes. As I have stated elsewhere in these comments, I believe that the NOEL for cholinesterase inhibition is the same for both sexes in the 2 year rat bioassay. Thus, no additional uncertainty factor is needed for this possible increased sensitivity because the RfD is based on the NOEL.

Question 2) What approach might be taken to estimate, from the data currently available, a correction factor to be applied to the NOEL derived from the Moeller and Rider study in male human subjects to afford equivalent protection for women?

Based on the possible difference in the extent of cholinesterase inhibition between male and female rats at the LOELs in the 2 year bioassay, the NOEL/LOEL range in female humans could potentially be lower. Alternatively, the male human study is by gavage; thus, the dietary NOEL/LOEL range in human males could potentially be higher. These uncertainties might be quantifiable based on an analysis of other data for this chemical, or perhaps related chemicals, but they do operate to cancel each other out. The end result would be that the expected human female dietary NOEL might be of the same relative value as the existing human male gavage NOEL.

I do not support the use of an additional factor to account for the potential differences in cholinesterase inhibition between sexes, because in rats, the NOELs for cholinesterase inhibition in the 2 year bioassay are the same.

Question 3) Should additional testing in animal models be required to further quantitate the gender specific disparity?

No. The NOELs for cholinesterase inhibition in both male and female rats are the same in the critical study.

VIII Cholinesterase Inhibition - Chronic Dog Study
Supporting documentation: DER 0s 1 and 4; References: B (p. 4), H, I and AA

Question: Knowing that the chronic dog study has no NOEL for cholinesterase inhibition and was considered unacceptable, should additional work, e.g. subchronic feeding study, be required to characterize cholinesterase inhibition in the dog?

We have a well characterized NOEL for cholinesterase inhibition of 4 mg/kg-day in rats of both sexes for 2 years, and a handle on where humans (the species of interest) are responding with cholinesterase inhibition from the Moeller and Rider study. With the use of an additional uncertainty factor of 3-fold for data base deficiencies applied to the rat NOEL, the RfDs from the human and rat information are quite comparable.

I do not see the need for additional testing in the dog.

External Peer Reviewer: Rolf Hartung, Ph.D., DABT

PEER REVIEW OF THE RfD AND EVALUATIONS BY THE U.S. EPA's HEALTH EFFECTS DIVISION FOR MALATHION

Reviewed by:

Rolf Hartung, Ph.D., DABT
Professor Emeritus of Environmental Toxicology
University of Michigan
3125 Fernwood Ave.
Ann Arbor, MI 48108-1955

May 29, 1998

CHARGE TO REVIEWERS FOR THE RFD, AND EVALUATION OF MALATHION

And Additional Questions

The reviewer is asked to review each of the DERs, with the exception of the DERs pertaining to mutagenicity, and determine if the end-points were appropriately chosen and if the studies were adequately run. Was the appropriate Uncertainty Factor used in the establishment of the RfD?

Answer:

The brief summaries of the experimental conditions that were provided are generally inadequate to determine whether the studies were appropriately conducted.

If a study was conducted using an established protocol, under careful and conscientious conditions, then the data generated by such a study are by my definition acceptable and probably useful. Judgments that are based primarily on *ex post facto* assessments of the data (e.g. was the MTD reached), should have no influence on the validity of the data, but only on their utility for a very specific purpose) (see Doc. #2).

The assessment of data cannot depend upon single pair-wise comparisons or upon differences of means or single values between experimental treatment groups and control groups. Real toxicological responses are inherently part of dose-response continua. Thus, effects noted at low levels of exposure should have counterparts of greater severity at higher dose levels. Furthermore, one would not expect to observe minute changes in responses associated with order of magnitude changes in dose levels. The only exception to the principle of stand-alone effects could occur for observations noted only at the highest dose level tested.

The assessment needs to incorporate the entire harmonized data set from all studies. It should not depend upon a search for single values, which are then treated without context.

In the case of DER #1, the selection of the plasma cholinesterase inhibition in male rats is not without problems. First of all, I would have liked to see the cholinesterase data displayed in tabular form, instead of in many paragraphs of potentially confusing text. Secondly, the dose levels between the observed LOEL and the NOEL are an order of magnitude (or more, based upon actual food intake) apart, making the estimate of the actual NOEL problematical. Mechanistically, the interest in plasma and erythrocyte cholinesterase inhibition relates to their association with the potential for inhibition of acetylcholinesterase in brain. Plasma cholinesterase has the fastest turn-over among the cholinesterases that are being discussed. Furthermore, plasma cholinesterase is synthesized in the liver, and can also be depressed as a consequence of liver damage. I find the discussion regarding the selection of plasma cholinesterase inhibition for the derivation of the RfD to be simplistic and superficial.

The level of plasma cholinesterase inhibition after Malathion (DER #1) needs to be reconciled with the observed level of plasma cholinesterase inhibition after exposure to the active compound, Malaoxon (DER #2).

The reviewer is asked to determine if the 2-year chronic feeding study in rats should be the correct selection as the Principal Study.

Answer:

The proposed RfD is based upon changes in plasma cholinesterase levels at 24 months, but not at earlier time points, in an extensive study in male F344 rats (DER #1), the effect was seen at much higher dose levels in female rats. The observed slope of the dose/response curve was very shallow. Human plasma cholinesterase activities regenerate fully within about 14 days, and red cell cholinesterase levels regenerate within about 100 days. Therefore the selection of the 24 month time point for the rat study does not make much sense.

Although this is an extensive study, it has been conducted in the wrong species. I clearly prefer using the existing human study, using the animal data as back-up.

The reviewer is asked, if the data base would be considered to be complete, and if the critical effects in each DER were appropriately chosen and used in the evaluation (ref. A), are there supporting studies which shore up the use of the principal study for the RfD?

Answer:

It would be desirable to have at least a brief discussion of the interrelations of the various cholinesterases at different sites, their functions, and their diagnostic utility in relation to OP poisoning.

As for completeness of the data base, in my opinion toxicological studies are inherently open-ended. Regardless of the existing mass of toxicological data, there are always additional questions that can be asked and the data base be further expanded, practically *ad infinitum*. Thus, there is **NO** compound that has a complete toxicological data base (I am including substances such as sugar and table salt here).

DETAILED REQUESTS FOR EVALUATION

I Hazard Identification/Acute Oral (One-Day)

Supporting documentation: DER #s 5, 6, 7, 9 and 19; References: A (pp. 3-5), B (pp.1-4), C, D, E, V and BB (pp. 12-14; 20-22)

Question 1): Do the rabbit developmental toxicity and developmental range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicologic consequence in either the maternal or the developing organisms?
Answer: DER #s 7 & 19 are repeated oral dose studies in rabbits. DER #19 is a range finding study of limited value which shows no significant effects at 50 mg/kg/day during gestation days 6-18. No significant effects were noted. DER #7 is a more complete study, also using oral doses during gestation days 6-18. This study shows increased resorption sites at 25 mg/kg. The available information is inconclusive whether a single dose, administered during a day of maximum sensitivity would be able to elicit the observed response, or whether cumulative dosing is required.

Question 2): Do data on matèrnal body weight and body weight gain now available in Appendix III of the rabbit developmental toxicity study alter the assignment of the LOEL/NOEL for the study, and do they influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxicologic effect?
Answer: The body weight and weight gain data can be interpreted as evidence of slight toxicological effects in the pregnant does at 50 mg/kg and higher. This evidence of maternal toxicity may be the basis for increased resorption sites at these dose levels.

Question 3): As presented in a published work in the open literature, single intraperitoneal doses as low as 50 mg/kg in the rat reportedly elicited a clear effect on avoidance performance while cholinesterase inhibition (erythrocyte) was observed at 100 mg/kg. Plasma and brain cholinesterases were also inhibited at 150 mg/kg. Cholinesterase inhibition and decrements in behavior were all very significant though transient effects: a) What level of confidence should be accorded this study?; b) What is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg can serve as the endpoint for acute dietary (one-day) risk assessment?; c) Are the data available in the developmental toxicity studies sufficiently reliable to discount the 10X safety factor required under FQPA?
Answer: Changes in some behavioral parameters that have a degree of correspondence to acetylcholinesterase, in particular to brain cholinesterase, would be expected. There is no requirement that the dose/response curves for acetylcholinesterase inhibition and for behavioral changes should coincide. A report by Kurtz (1977) indicates that neonate rats exhibit decrements in avoidance behavior more sensitively than adults. The biggest problem with these studies is the use of intraperitoneal dosing as a surrogate for environmentally realistic and relevant routes of exposure, which cannot be defended quantitatively. Both the distribution patterns and the pharmacokinetics associated with intraperitoneal dosing are sufficiently different from dosing by ingestion, inhalation, or percutaneous routes, to render most quantitative assessments to be very doubtful, and to create reservations even for qualitative assessments. In most cases the i.p.

route leads to more rapid absorption, and therefore higher peak blood levels than environmentally relevant exposures.

The study by Pope and Chakraborti (1992) reports higher sensitivity for cholinesterase inhibition in neonates as compared to adults. However, the study was conducted with parathion, methyl parathion and chlorpyrifos, not malathion. In the case of the mammalian toxicity of the more complex OPs (such as the phosphorodithioates), the toxicity is the result of the balance between activating and detoxifying pathways. Both types of pathways may not be fully developed in the neonate, so that it is not useful to hypothesize on the balance among these pathways for malathion in neonates based upon inferences derived from other OPs.

The additional 10X safety factor under FQPA originates in part from the inescapable higher metabolic rates and surface areas of children, which result in increased doses on a body weight basis under equivalent environmental conditions when compared to adults. The available information does not support the hypothesis that neonates are less sensitive than adults on a body weight basis. Therefore, the available information does not support deletion of the 10X FQPA safety factor.

II Determination of Susceptibility, Reproductive Toxicity

Supporting documentation: DER: # 5; References: A (pp. 15-16), B (pp. 3-4), F. G and BB (pp. 12-14; 16-17; 20-22)

Question 1) Can the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation reproduction study in the Sprague-Dawley rat be dismissed as "...not a true indication of increased sensitivity of offspring..." for the reasons stated in the Hazard ID Committee report?

Answer:

I concur with the reasoning of the Committee. But I do not concur with the ultimate conclusions with respect to safety (uncertainty) factors to be used. Whether neonates are more sensitive to a given dose of pesticide (mg/kg basis) is an important, but not the only issue. The newborn are known to have higher food intakes (on a body weight basis) than adults. The higher intake is the consequence of higher metabolic rates, due to increased heat loss determined by surface area/body weight of the neonate, and due to increased food intake due to growth requirements. For foods with equal pesticide residues the child will acquire a higher dose of pesticides than the adult (see NRC, 1993; Pesticides in the Diets of Infants and Children). Given this circumstance, it is necessary to demonstrate that the neonate is less sensitive (not equal to) than the adult on a mg/kg basis, or that food pesticide residues are being managed in such a way as to restrict the quantities of residues likely to be found in the diet of children as compared to adults.

Question 2) In the absence of assessments of cholinesterase inhibition and behavioral effects testing in adult and young animals in reproduction studies, can the data obtained in the FIFRA guideline study be considered adequate to address the question of whether young or mature animals are the more sensitive to malathion?

Answer:

The comparison of relative sensitivities is only meaningful when data exist that were collected under comparable conditions. It is obviously possible to make many comparisons, as long as comparably derived data exist. However, because toxicity testing is inherently open ended, a question of this type can never be answered with certainty in detail, although general comparisons are possible.

Question 3) Does this two-generation reproduction study provide the reliable evidence of no increased sensitivity in pups when compared to adults, as required under FQPA, to discount the 10X safety factor imposed by FQPA as additional protection for infants and children?
Answer:

That is not the correct question, the appropriate question should be whether this study provides clear evidence of less sensitivity among pups as compared to adults - for reasons cited elsewhere in this report.

III Hazard Identification/Chronic Dietary (RfD)

Supporting documentation: DERs: #s 1 and 10; References: A (pp. 5-6), B (pp. 4-5), H. I, N (p. 1 6), R and Y.
Question 1) Given the evidence of a post 3 months recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, can 50 ppm be concluded to have been a NOEL for the first three months of testing?
Answer:

No. Once the malathoxon-acetylcholinesterase bond is established in RBCs, the rate of recovery is identical to the red cell replacement rate, which is approximately 1%/day.

Question 2) Alternatively, do these findings suggest flawed cholinesterase methodology, and if so, what corrective measure could be pursued?
Answer:

This requires an analysis of the detailed cholinesterase methodology. The cholinesterases as well as many other esterases are surprisingly non-specific. Which substrates were used ? What was the pH and temperature? Were substrate and product inhibition avoided ? (See G. B. Koelle, 1963. Cholinesterases and anticholinsterase agents. Handbuch der experimentellen Pharmakologie, Ergänzungswerk, Vol. 15. Springer Verlag, Berlin)

Question 3) Should 4 mg/kg/day, the NOEL for plasma cholinesterase inhibition in males, be supported as a replacement for human data previously relied upon in establishing the RfD, or should additional testing be required in the rat to identify a NOEL for cholinesterase inhibition, particularly in females?
Answer:

No. The human is the correct species of concern. Substituting a rodent introduces many more uncertainties than those produced by minor deficits in the analysis of chemical purity or concerns about statistical precision.

Question 4) Given that an explanation exists for a greater sensitivity of humans than rats with respect to cholinesterase inhibition from malathion exposure (i.e. the lack of carboxylesterase in human plasma) should a 10X safety factor applied to the rat data to allow for "uncertainties" in interspecies variability be considered adequate if the rat data is to be used in deriving the RfD?

Answer:

Look at what you are doing! Here you are willing to accept a study for which you are also willing to mess around with another factor of 10X, just because the statistical data are neater. In the process you are willing to discount human data, even though it is extremely unlikely that the equivalent statistical uncertainties for the human will reach anywhere close to 10X !

Question 5) Further, given that the RfD based on human data (0.023 mg/kg/day) is lower than that derived from the rat data (0.040 mg/kg/day) and that an explanation exists for a greater sensitivity for humans, should the RfD based on human data be retained?

Answer:

YES

Question 6) Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?

Answer:

See previous discussions related to the 10X FQPA safety (uncertainty?) factor.

IV Subchronic Inhalation Study

Supporting documentation: DERs: #s 1, 2 and 13; References: A (pp. 9-11), B (pp. 5-6), J. N (p 12) and O

Question 1) Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngeal degeneration/hyperplasia supportable?

Answer:

This fine-tuning is unwarranted because of major species differences in exposure scenarios. In whole body exposures of rodents we deal with a direct nasal inhalation, an inhalation filtered through fur as rats assume a characteristic protective fetal position during the exposure, and a direct ingestion as rats seek to clean their fur by licking it. This is not comparable to the exposure scenario of the human applicator, the bystander, or the consumer.

The unique nasal responses of rats have been discussed previously during the assessment of the inhalation toxicity of formaldehyde.

Question 2) A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not identify NOELs for cholinesterase inhibition or histopathological findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to invoke an uncertainty factor for acute risk assessment (i.e. 1-7 days) on the basis of cumulative effects?

Answer:

The same objections raised for Question IV-1 still apply here.

Question 3) Should another study be required to identify the NOEL for the end points in question?

Answer:

Not with rats on these issues.

Question 4) Given the findings of nasal and laryngeal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalation route (e.g., inhalation exposure for first 90 days of a two year study)?

Answer:

There are so many questions regarding the validity of whole body exposures that their validity for human health risk assessments will remain questionable. Nose-only studies impose so many additional stresses on the rat, that the interpretation of any results also becomes seriously confounded.

Question 5) Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?

Answer:

No

V Acute Neurotoxicity Study (Retinal Rosettes)

Supporting documentation: DER #s 9 and 10; References: L, M and P (pp. 1-2)

Question 1) Should retinal histopathology data be submitted for rats in the intermediate dose group?

Answer:

Yes, for qualitative analysis, only. The reason for this is that either the dosing schedule or the cholinesterase analyses were deficient in this study. But, retinal detachments as potential end-points are sufficiently serious to merit following up on the present anecdotal finding.

Question 2) Should histopathology slides be submitted for independent examination by the Agency's pathologist (for anatomic features comparison between control and treatment group lesion) as called for in the Data Evaluation Record (DER) for this study (a relatively simple request)?

Answer:

They should be examined. Whether by the mechanism in question 1 or 2 does not particularly concern me at this time.

VI Subchronic Neurotoxicity Study

Supporting documentation: DER #s 10, 11; References: D, P (pp. 3-4), Q. S. T. U and BB (pp. 1 2-14; 1 6-1 7; 20-22)

Question 1) Given the contrast between the NOEL of 1575 mg/kg/day (HDT) for female rats on neurotoxicity end points in this FIFRA Guideline study and that of the LOEL of 38 mg/kg/day (LDT) in the published work on a different set of neurotoxicity parameters, does the published work provide adequate reason or evidence to require a developmental neurotoxicity Guideline study or another neurotoxicity study that embraces learning/memory, EEG, EMG, and possibly other neurotoxicity parameters not covered in the subchronic neurotoxicity Guideline study?
Answer:

Neurotoxicity studies are generally fairly easy to conduct, but they tend to present major difficulties in the selection of proper controls and in the interpretation of the results, especially between different species. The studies in DER #10 and DER #11 show no behavioral effects at dose levels significantly above dose levels associated with plasma cholinesterase inhibition, but they do show abnormalities in EEG and EMG recordings after 90 days of exposure.

Historically, Russian scientists have relied heavily upon neurophysiological measurements in the assessment of toxicity. These studies have generally not been accepted in western nations, mainly because of difficulties with interpreting the data. If this issue is to be reconsidered, then it would require an in-depth assessment of the the under-lying science, and its utility for developing acceptable exposures. Furthermore, such an approach would need to be extended to most pesticides.

The spread between simple behavioral responses and cholinesterase inhibition argues against a need for further study. But, the sensitivity of behavioral studies can vary significantly, depending upon the end-point that is measured.

I am not familiar with the full range of studies that could be asked for under a 'developmental neurotoxicity Guideline' study. If this is an unrestricted list of test methods, then it should be subjected to an assessment of interpretability and utility prior to requiring its execution.

Question 2) If the neurotoxicity findings in the published study are considered inadequate to trigger the additional Guideline testing, what criteria from published work, short of those upon which regulations could be directly based, might serve in that capacity? (Note: Moeller and Rider (1962), a journal publication with attendant Guideline deficiencies, has served for decades as the basis for a regulateable end point (RfI)) for malathion, while the publication in question here is only being put forth as sufficiently definitive to require a study in the FIFRA Guidelines heretofore not performed.)
Answer:

There is a major difference between the Moeller and Rider study and DER #10 & 11. The Moeller and Rider study is conducted in the proper species, and it addresses a diagnostic end-point that is known to be mechanistically related to the toxicity of OPs. DER #11 (Dési et al.) is based upon EEG frequency band ratios and upon the EMG frequency of unit potentials. I see no difference in the difficulties of interpretation of this study and a voluminous body of Russian neurotoxicological literature that we have rejected historically.

VII Cholinesterase Inhibition - Enhanced Sensitivity of Females
Supporting documentation: DER #s 1 - 3, 9, 10, 12 and 13; References: W. X, Y. Z and CC

Question 1) Does the malathion data base support a conclusion that females are the more sensitive gender with respect to cholinesterase inhibition by this organophosphate?
Answer:

The data were not assembled in a way that would allow ready assessment of this question. The emphasis in the data presentation for the present review centers on NOELs and LOELs, etc., not on the actual dose response data (corrected for actual consumptions), which should be plotted and tested for differences. The available information should be found in the supporting data for DER #1 for rats, DER #3 for mice, DER #4 for dogs, DER #9 for rats, DER #10 for rats, DER #12 for rats (percutaneous absorption, use with caution). These data, especially as summarized in Refs. W, Y, and Z, indicate that females compared to males are in some cases approximately equally sensitive to cholinesterase inhibition, and in other cases are more sensitive. I was unable to find any rigorous statistical comparisons of the underlying dose/response relationships.

DER #2 does not contribute to this question, it was derived for malaoxon.

Question 2) What approach might be taken to estimate, from the data currently available, a correction factor to be applied to the NOEL derived from the Moeller and Rider study in male human subjects to afford equivalent protection for women?
Answer:

I am not aware of any direct data that show that the human female is more sensitive to cholinesterase inhibition for equivalent doses than males are. Therefore, the simplest first step is to explore whether the existing data indicate whether gender-mediated sensitivities are universal across the species that have been tested to date. If so, then what is the magnitude (ratio) of the difference?
If a ratio can be discerned and defended, then it can be applied to the data applicable for the human male.

Question 3) Should additional testing in animal models be required to further quantify the gender specific disparity?
Answer:

1.- Do a thorough analysis of existing data first, as suggested for question 2.
2.- Add another species, if necessary.
3.- If possible, expand the Moeller study to include females.

VIII Cholinesterase Inhibition - Chronic Dog Study

Supporting documentation: DER #s 1 and 4; References: B (p. 4), H. I and AA

Question: Knowing that the chronic dog study has no NOEL for cholinesterase inhibition and was considered unacceptable, should additional work, e.g. subchronic feeding study, be required to characterize cholinesterase inhibition in the dog?
Answer:

No, the study is perfectly adequate for the detailed analysis of the dose/response curves

for cholinesterase inhibition. As a matter of fact, the elusive NOEL is practically useless for a rigorous dose/response analysis to explore gender-mediated or species-mediated differences in sensitivity.

ATTACHMENT 2: HIAR REPORT OF 12/17/97

HED Doc. No. 012440

Date: **December 17, 1997**

MEMORANDUM

SUBJECT: ***MALATHION:*** - Report of the Hazard Identification Assessment Review Committee.

FROM: Jess Rowland
Branch Senior Scientist,
Science Analysis Branch, Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Chairman,
Hazard Identification Assessment Review Committee
Toxicology Branch II, Health Effects Division (7509C)
And
Mike Metzger, Co-Chairman
Hazard Identification Assessment Review Committee
Reregistration Action Branch 2, Health Effects Division (7509C)

TO: Al Nielsen, Branch Senior Scientist
Reregistration Branch 2
Health Effects Division (7509C)

PC Code: 057701

On November 6, 1997, the Health Effects Division's Hazard Identification Review committee evaluated the toxicology data base, selected doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure risk assessments, and addressed the sensitivity of infants and children from exposure to Malathion as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

Committee Members in Attendance

Members in attendance were Karl Baetcke, William Burnam, George Ghali, Karen Hamernik, Susan Makris, Nancy McCarroll, Mike Metzger, Kathy Raffaele, John Redden, Jess Rowland and Clark Swentzel. Member in absentia: Melba Morrow. Data was presented by Ed Budd of Registration Action Branch 2.

HED staff members also participating at the meeting were: Brian Dementi, Toxicology Branch 1, William Sette, Science Analysis Branch and, Paula Deschamp (Exposure Scientist), Diana Locke (Risk Assessor), and Pauline Wagner (Chief, Reregistration Action Branch 2).

Data Presentation: _____
Ed Budd, M.S

Report Preparation: _____
Jess Rowland, M.S

I. INTRODUCTION

On November 6, 1997, the Health Effects Division's Hazard Identification Review committee evaluated the toxicology data base to select the doses and endpoints for acute dietary, chronic dietary (RfD) as well as occupational and residential exposure risk assessments, and addressed the enhanced sensitivity of infants and children from exposure to Malathion as required by the Food Quality Protection Act (FQPA) of 1996.

II. HAZARD IDENTIFICATION

A. Acute Dietary (one-day)

Study Selected: Range-Finding Developmental Toxicity - Rabbit and Developmental Toxicity - Rabbit §83-3

MRID Nos. 00152569 (Range Finding) 40812001 (Main Study)

Executive Summary: Range Finding: In a Range-Finding study, pregnant New Zealand white rabbits (5/group) received oral administration of Malathion (92.4%) in corn oil at doses of 0, 25, 50, 100, 200, or 400 mg/kg/day on Gestation Days (GD) 6-18. No mortalities or clinical signs were observed at 25, 50 or 100 mg/kg/day. At 200 mg/kg/day, 2 does died, 1 on GD 11 (5 days after dosing) and another on GD 17 (11 days after dosing). At 400 mg/kg/day, 4 does died, 1 on GD 7, 1 on GD 8 and 2 on GD 9. Cholinergic signs of toxicity seen at 200 and 400 mg/kg/day included tremors, decreased activity and salivation. External examinations of the fetuses did not indicate any gross abnormalities. For Maternal Toxicity, the NOEL was 100 mg/kg/day and the LOEL was 200 mg/kg/day based on mortality and clinical signs.

Main Study: In a prenatal developmental toxicity study, pregnant New Zealand White rabbits (20/group) received oral administration of Malathion (92.4%) in corn oil at doses of 0, 25, 50, or 100 mg/kg/day on gestation days 6-18. Mortality in 2 does at 100 mg/kg/day were attributed to dosing (gavage) errors. For Maternal Toxicity, the NOEL was 25 mg/kg/day and the LOEL was 50 mg/kg/day based on reduced mean body weight gains during treatment. During the dosing period (Days 6-18), mean body weight gains were 0.19, 0.06, -0.03 and -0.03 kg at 0, 25, 50 and 100 mg/kg/day, respectively. Anorexia and soft stools were seen at all doses including the controls, but occurred at a slightly higher incidence at 100 mg/kg/day. For Developmental Toxicity, the NOEL was 25 mg/kg/day and the LOEL was 50 mg/kg/day based on a slightly increased incidence of mean resorption sites per dam.

Dose and Endpoint for Risk Assessment: NOEL of 50 mg/kg/day. The Committee selected this dose based on a weight-of-the-evidence consideration from the Range-Finding and the Main Study as well as pertinent information from other studies.
Comments about Study and Endpoint: In the Range-Finding study no deaths occurred at 100 mg/kg/day. Death attributable to a single dose (i.e., the period of exposure of concern)occurred only in 1 doe on the first day of dosing (GD 7) at 400 mg/kg/day.

At 200 mg/kg/day deaths occurred only after multiple doses (i.e., GD 11 and 17)]. No treatment-related mortality was seen in the main study. None of the clinical signs (anorexia and soft stool) seen in both studies were attributable to a single exposure.

In the Main Study, the decrease in mean body weight gain in does at 50 mg/kg/day (LOEL) observed during the dosing period was not attributable to a single dose but rather to multiple doses. It should be noted no mortalities, clinical signs or decreases in body weight gain were seen at the same dose (50 mg/kg/day) in the Range-Finding study. Thus, toxicological endpoints (e.g., death, clinical signs) attributable to a single dose were not observed at 50 mg/kg/day. The increase in resorption sites/dam at 50 mg/kg/day was not considered to be an appropriate endpoint because the incidence was only slightly increased and was considered by the Committee to be of no meaningful toxicological significance with respect to acute dietary risk assessment. Based on a weight-of-the-evidence consideration from the Range-Finding and the Main Study and other pertinent information from other studies on Malathion, the Committee determined that a NOEL of 50 mg/kg/day is appropriate for acute dietary risk assessment.

The Committee did not consider the acute neurotoxicity study in rats (MRID No. 43146701) to be appropriate because of low confidence in the assessment of cholinesterase activity. In rats given a single oral dose of Malathion at 0, 500, 1000 or 2000 mg/kg, plasma and erythrocyte cholinesterase were inhibited in both sexes at 2000 mg/kg on Day 7, a finding which was sustained, in females only on Day 15. Also, there was equivocal inhibition of plasma cholinesterase in females at 500 and 1000 mg/kg which was characterized by a poor dose response. No inhibition of brain cholinesterase activity was seen in either sex at any dose level. Thus the lack of dose response and a clear NOEL for this biomarker constituted an inherent weakness of this study since inhibition of cholinesterase activity was seen in other studies among various species (rats, and dogs) at much lower doses.

This risk assessment is required.

Acute Dietary Risk Assessment: The Committee determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be removed.** For acute dietary risk assessment, **a Margin of Exposure (MOE) of 100 is adequate** for the protection of the general U.S. population including infants and children from acute exposure to Malathion. A MOE of 100 is adequate because.

(I) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

(ii) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.

(iii) The toxicology data base is complete and there are no data gaps.

B. Chronic Dietary [Reference Dose (RfD)]

Study Selected: Combined chronic Toxicity/carcinogenicity -Rat Guideline §83-5

MRID No. 43942901

Executive Summary: In a combined chronic toxicity/carcinogenicity study, groups of Fischer 344 rats (90/sex/dose) were fed diets containing Malathion (**97.1%**) at 0, 100/50, 500, 600 or 12000 ppm (equivalent to 0, 4, 29, 359 or 739 mg/kg/day in males and 0, 5, 35, 415 or 868 mg/kg/day in females, respectively) for up to 24 months. The low dose of 100 ppm was reduced to 50 ppm after 3 months due to inhibition of erythrocyte cholinesterase activity in females. For chronic toxicity, the NOEL was 50 ppm (4 mg/kg/day) and the LOEL was 500 ppm (29 mg/kg/day) based on inhibition of plasma cholinesterase activity in males at 24 months.

Dose and Endpoint for establishing the RfD: NOEL=4 mg/kg/day based on significant inhibition of plasma cholinesterase activity at 29 mg/kg/day (LOEL).

Uncertainty Factor (UF): An UF of 100 was applied to account for inter (10 x)-and intra-(10 x) species variation.

$$\text{RfD} = \frac{\text{4 mg/kg/day (NOEL)}}{100} = \textbf{0.04 mg/kg/day}$$

Comments about Study and Endpoint: The RfD derived from the use of the NOEL and endpoint from the above animal study and an Uncertainty Factor of 100 is supported by a comparable RfD that could have been derived from the use of the NOEL from a human study and an Uncertainty Factor of 10.

In a 1962 study conducted with male human volunteers, Malathion (**purity not known**) was administered by gelatin capsule once each day to groups of 5 healthy male volunteers ranging in age form 23 to 36 years. Based on an assumed body weight of 70 kg, the dosage regimen was 0.11 mg/kg/day for 32 days, 0.23 mg/kg/day for 47 days and 0.34 mg/kg/day for 56 days. Plasma and erythrocyte cholinesterase activities were determined twice weekly before, during and after administration. Some of the volunteers were also given another test material (EPN) alone or in combination with various doses of Malathion over the course of the study. No clinical signs or symptoms of toxicity were observed at any dose level at any time. The NOEL was 0.23 mg/kg/day and the LOEL was 0.34 mg/kg/day based on inhibition of plasma and erythrocyte cholinesterase.

When the **NOEL** of 4 mg/kg/day **from an animal** study is used in conjunction with

an **Uncertainty Factor of 100** (10 x for inter-species and 10 x for intra-species variations), the **RfD** derived **is 0.04 mg/kg/day.**

When the **NOEL** of 0.23 mg/kg/day **from a human study** is used in conjunction with an **Uncertainty Factor of 10** (for intra-species variation), the **RfD** derived **is 0.02 mg/kg /day**.

The Committee decided to use the animal study instead of the human study for deriving the RfD for the following reasons: 1) low confidence in the human study due to the use of only one sex (males), unknown purity of Malathion, and the unavailability of raw data for proper evaluation); 2) the completeness of the animal toxicology data base, known purity (97.1%) of Malathion tested and the NOEL of the 2-year study supported by the results in a 13-week neurotoxicity study in rats in which the NOEL for inhibition of cholinesterase activity was also 4 mg/kg/day in both males and females.

Chronic Dietary Risk Assessment: The Committee determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be removed.** For chronic dietary risk assessment, **a UF of 100 is adequate** for the protection of the general U.S. population including infants and children from chronic exposure to Malathion. A UF of 100 is adequate because.

(I) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

(ii) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.

(iii) The toxicology data base is complete and there are no data gaps.

C. Occupational/Residential Exposure

1. Dermal Absorption

Study: Published Study (Feldman, RJ and Maibach, HI. (1970)

Executive Summary: In this dermal absorption study in humans, 14C-radiolabeled Malathion (dissolved in acetone) was applied to a 13 sq cm circular area on the ventral surface of the forearms of 7 subjects at a rate of 4 ug/sq cm. The skin sites were not protected. All urine was collected for 5 days and assayed for radioactivity in a liquid scintillation counter. Dermal penetration of Malathion through the skin was estimated by calculating the

total amount of radioactivity excreted in the urine in 5 days. A mean of 7.84% ±2.71% (SD) of the applied dose of radioactivity was recovered in the 5 day urine, indicating a dermal absorption rate of approximately 5% to 10% over a 5 day period. Based on the above, the Committee concluded that the dermal absorption rate is about 10%.

Dermal Absorption Factor: A dermal absorption factor of 10% should be used for converting oral dosing to dermal dosing.

Comments about Study: The dermal absorption rate of 10% established in the human study is supported by the dermal absorption (DA) calculated by comparing the NOELs or LOELs in the oral developmental toxicity study and the 21-day dermal toxicity study in the same species (rabbits) as shown below

Type of Study	NOEL	LOEL	Estimated DA based on NOELs	Estimated DA based on LOELs
Main Study-Developmental	25	50	25÷1000 = 2.5%	50÷1000=5%
Range-Finding-Developmental	100	200	100÷1000=10%	200÷1000=20%
21-Day Dermal Toxicity	1000	>1000		

Additional support for the dermal absorption rate of 10% in humans is provided in a study by Castles and Reddy. In that study, human percutaneous absorption was determined for Malathion (neat), Ortho Malathion 50 (50% Malathion in Xylene), Ortho Malathion diluted to 1% in water and Ortho Malathion diluted in 10% water when applied to the forearm. The mean doses applied were 0.8, 0.9, 0.032 and 1.13 mg/sq cm. respectively. Mean absorption, based on urinary excretion of label, for a 24 hour exposure was 7.2, 5.6, 15.0 and 5.5, respectively (Castles and Reddy, January, 1993; Tox.Doc.No. 011314).

2. Short-Term Dermal - (1-7 days)

Study Selected: 21-Day Dermal Toxicity - Rabbit Guideline §82-2

MRID No 41054201

Executive Summary: Groups of New Zealand White rabbits (6/sex/dose) received 15 repeated dermal applications of Malathion (94%) at 0, 50, 300 or 1000 mg/kg/day, 6 hours/day, 5 days/week over a three week period. Except for one death at the high dose due to acute mucoid gastroenteritis, no mortality occurred. No treatment-related effects were seen in body weight, body weight

gain, food consumption, clinical signs, hematology or clinical chemistry parameters, organ weights and gross or histopathology. Slight dermal irritation was seen at application sites. The overall NOEL was 50 mg/kg/day and the LOEL was 300 mg/kg/day based on significant inhibition of plasma and red blood cell cholinesterase activity in both sexes and in the brain of females.

Dose and Endpoint for Risk Assessment: NOEL = 50 mg/kg/day based on significant inhibition of plasma, red blood cell and brain cholinesterase activity at 300 mg/kg/day (LOEL).

Comments about Study and Endpoint: Inhibition of cholinesterase activity following oral administration was also observed following dermal applications, the route of exposure of concern.

This risk assessment is required.

3. Intermediate-Term Dermal (7 Days to Several Months)

Study Selected: 21-Day Dermal Toxicity - Rabbit Guideline §82-2

MRID No 41054201

Executive Summary: See Short-Term

Dose and Endpoint for Risk Assessment: NOEL=50 mg/kg/day based on significant inhibition of plasma, red blood cell and brain cholinesterase activity at 300 mg/kg/day (LOEL).

Comments about Study and Endpoint: The Committee selected this dose for this risk assessment, since the NOEL of 50 mg/kg/day established following dermal exposure in the 21-day dermal study is supported by the NOEL of 4 mg/kg/day established following oral exposure in the 13-week neurotoxicity study in rats when a dermal absorption factor of 10% is applied. In both studies (i.e. via both routes), the LOEL was based on a common toxicological endpoint, inhibition of plasma, red blood cell and brain cholinesterase activity.

In a subchronic neurotoxicity study (MRID No. 43269501), Sprague-Dawley rats received dietary administration of Malathion at 0, 4, 352 or 1486 mg/kg/day to males and at 0, 4, 395 or 1575 mg/kg/day to females for 13 weeks. For cholinesterase activity, the NOEL was 4 mg/kg/day in both sexes and the LOEL was 352 mg/kg/day in males and 395 mg/kg/day in females

based on inhibition of plasma and erythrocyte cholinesterase activity in both sexes and on inhibition of brain cholinesterase activity in females.

Application of a dermal absorption rate of 10% to the oral NOEL of 4 mg/kg/day yields a comparable dermal dose of 40 mg/kg/day (4 mg/kg/day÷10%DA = 40 mg/kg/day). Thus the 40 mg/kg/day is analogous to the 50 mg/kg/day NOEL observed in the 21-day dermal study in rabbits based on the same toxicological endpoints.
This risk assessment is required.

4. Long-Term Dermal (Several Months to Life-Time)

Study Selected: Combined Chronic Toxicity/Carcinogenicity - Rat §83-5

MRID No 43942901

Executive Summary: See Chronic Dietary

Dose and Endpoint for Risk Assessment: NOEL=4 mg/kg/day based on significant inhibition of plasma cholinesterase activity at 29 mg/kg/day (LOEL).

Comments about Study and Endpoint: This dose and endpoint was used in establishing the RfD. Since an oral dose was selected, a dermal absorption rate of 10% should be used in dermal risk assessments. When a dermal absorption rate of 10% is applied to the oral NOEL of 4 mg/kg/day, a comparable dermal dose of 40 mg/kg/day is obtained (i.e., 4 mg/kg/day ÷10% DA = 40 mg/kg/day).

This risk assessment is required.

5. Inhalation Exposure (Any-Time period)

Study Selected: 90-Day Inhalation -Rat Guideline: 82-4

MRID No 43266601

Executive Summary: In a subchronic inhalation study, groups of Sprague-Dawley rats (15/sex/concentration) were exposed in whole body inhalation chambers to Malathion (96.4%) at aerosol concentrations of 0.1, 0.45, or 2.01 mg/L for 6 hours/day, 5 days/week for 13 weeks . Treatment had no effects on survival, body weights or food consumption. Cholinergic signs observed at 2.01 mg/L and sporadically in a few animals at the lower doses included red staining of the urogenital areas, excess salivation and ungroomed oily fur.

Treatment-related histopathological lesions were seen in the nasal cavity and the larynx of both sexes of rats at all concentrations tested. The lesions in the nasal cavity were characterized as slight to moderate degeneration and/or hyperplasia of the olfactory epithelium which was locally extensive. The lesions of the larynx were characterized as epithelial hyperplasia, with squamous keratinization occurring in some rats. In addition, the olfactory/respiratory epithelial junction was severely affected in most animals. For systemic toxicity, a NOEL was not established and the LOEL was 0.1 mg/kg/day based on histopathologic lesions of the nasal cavity and larynx. Inhibition of plasma and red blood cell cholinesterase activity was seen in female rats at all concentrations. In male rats, inhibition of cholinesterase activity was observed in plasma at 2.01 mg/L and in red blood cells at $\geq$ 0.45 mg/L. Inhibition of brain cholinesterase activity was seen only at the highest concentration. For overall cholinesterase inhibition, a NOEL was not established for plasma and red blood cells; the LOEL was 0.1 mg/L. For inhibition of brain cholinesterase, the NOEL was 0.45 mg/L and the LOEL was 2.01 mg/L.
Dose and Endpoint for Risk Assessment: LOEL = 0.1 mg/L based on inhibition of plasma and red blood cell cholinesterase activity and histopathological lesions of the nasal cavity and larynx at the lowest concentration tested.

Comments about Study and Endpoint: Since this is the only inhalation study that is available in the toxicology data base, the LOEL from this study will be used for Short-, Intermediate-and Chronic inhalation risk assessments.

This risk assessment is required.

D Margin of Exposure for Occupational/Residential Exposures:

1. MOE for Dermal Exposures

For Short-, Intermediate- and Long-Term dermal exposures **a MOE of 100 is adequate** for occupational and residential exposures to Malathion via the dermal route because:

(I) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

(ii) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.

(iii) The toxicology data base is complete and there are no data gaps.

2. MOE for Inhalation Exposures

The dose (0.1 mg/L) selected for inhalation risk assessments is a LOEL and thus ordinarily would necessitate the use of an additional Uncertainty Factor (UF) for risk assessments (under FIFRA). For Malathion, however, an additional UF of 3 should be applied only for Intermediate and Long-Term but not for Short-Term exposure risk assessments.

The Committee determined that an additional UF of 3 is not required for Short-Term risk assessments because the toxicological endpoints (inhibition of plasma and red blood cell cholinesterase activity and histopathological lesions) seen at the lowest concentration tested are considered to be cumulative effects (the result of multiple dosing) and are not expected to occur after 1-7 days of treatment (the Short-Term exposure period of concern). In the acute neurotoxicity study, no cholinesterase inhibition was seen after a single oral dose except at a very high dose. In subchronic and chronic studies via the oral route, inhibition of cholinesterase activity has been observed only after repeated dosing with Malathion.

Therefore, the Committee determined that **a MOE of 100 is adequate** for **Short-Term exposure** risk assessments but **a MOE of 300 is required** for **Intermediate- and Long-Term exposure** risk assessments for exposures to Malathion via inhalation. The additional UF of 3 is applied under FIFRA because a LOEL was used for these risk assessments. No FQPA factors are required since there was no indication of increased sensitivity in the offspring of rats or rabbits in prenatal exposure studies on Malathion.

E. CLASSIFICATION OF CARCINOGENIC POTENTIAL

On September 24, October 8 and 15, 1997 HED's Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of Malathion. The CPRC reviewed the following studies: 1) Carcinogenicity study in B6C3F1 mice with Malathion; 2) Combined chronic toxicity/carcinogenicity study in Fischer 344 rats with Malathion; and 3) Combined chronic toxicity/carcinogenicity study with Malaoxon in Fischer 344 rats. The CARC recommended re-evaluation of certain tissues/slides from these studies since an assessment on the relevancy of observed tumors to treatment could not be made due to the absence of critical histopathological data. The CARC re-affirmed the current classification of Malathion as a **Group D** Carcinogen - Not Classifiable as to Human Carcinogenicity (Memoradum: J. Rowland SAB to M. Ioannou, TB2, November 3, 1997).

F. MUTAGENICITY

(I). Gene Mutation:

In a *Salmonella typhimurium/Escherichia coli* reverse gene mutation assay, Malathion (95.2%) was non-mutagenic when tested at concentrations up to 5000 µg/plate (highest dose tested) with or without S9 activation (MRID No. 40939302).

(ii). Chromosome Aberrations:

In an *in vivo* bone marrow cytogenetic assay, Malathion (94%) was negative following oral doses at 500-2000 mg/kg to male and female Sprague-Dawley rats. A dose-related reduction in mitotic indices (MIs) was seen in the females of all treatment levels at 24 hours. Reduced MIs were also recorded for high-dose males and females at 48 hours (MRID No. 41451201).

(iii). Other Mutagenic Effects:

In an *in vitro* primary rat hepatocytes unscheduled DNA synthesis (UDS) assay, Malathion (94%) was negative up to cytotoxic levels ($\geq$ 0.12 µL/mL; $\approx$ 150 µg/mL).(MRID No. 41389301).

(iv). Other Information:

Under the pre-1991 guidelines, the three acceptable studies *S.typhimurium/E. coli* reverse gene mutation assay, *in vivo* bone marrow cytogenetic assay in rats, and unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes (UDS)] satisfy the minimum requirements in the three major categories of genetic testing. The acceptable studies were negative. However, an open literature review of mutagenicity studies on Malathion and Malaoxon, a metabolite formed by oxidation, was prepared for the Carcinogenicity Peer Review of Malathion held on February 7, 1990 (see Memorandum from K. Dearfield to J. Edwards, 1990).

In addition, the mutagenicity potential of Malathion was again evaluated by HED's CPRC on September 24 and October 8 and 15, 1997. The overall assessment indicated that there is overwhelming confirmation from the published literature demonstrating that Malathion is genotoxic, producing structural damage to chromosomes *in vitro* and in whole animal studies with mice and hamsters. Similar conclusions were reached by Flessel et al., (1993) in the genetic toxicology review prepared for the California Department of Health Services. No assays with germinal cells have been submitted to the Agency. However, Malathion was negative in *Drosophila melanogaster* sex-linked recessive lethal assays, mouse dominant lethal assays and spermatogonia and/or spermatocyte cytogenetic assays. A questionable clastogenic response was reported in mouse spermatocytes following subacute exposure to commercial grade Malathion (Salvadori *et al.*, 1988). Nevertheless, the data from reproduction toxicity (MRID No. 41583401) and developmental studies

(00152569, 41160901) and epidemiological surveys of pregnant women exposed to Malathion (Arevalo *et al.*, 1987; Spielman, 1986; Grether, *et al.*, 1987) do not suggest adverse heritable effects. The Committee concluded, therefore, that requiring studies in germinal cells was not warranted.

No mutagenicity studies have been submitted to the Agency on Malaoxon. The consensus opinion from the above cited reviews of the open literature is that Malaoxon is not mutagenic in bacteria but is positive without S9 activation in the mouse lymphoma assay forward gene mutation assay. Malaoxon was not clastogenic in cultured Chinese hamster ovary (CHO) cells; however, the findings from the mouse lymphoma assay suggest that Malaoxon may induce both gene mutations and chromosome aberrations. Nonactivated Malaoxon also caused SCEs in independently performed investigations with CHO cells.

(iv). Conclusions:

The positive mutagenicity studies with Malathion support the evidence of liver tumor induction in male and female mice. Based on the overall results, there is a clear concern for somatic cell mutagenicity. No further testing is required since all mutagenicity issues have been addressed.

G. Recommendation for Aggregate Exposure Risk Assessments

For aggregate exposure risk assessment, the MOEs derived for the oral, dermal and inhalation exposures may be combined to obtain a total MOE since a common toxicological endpoint (i.e., cholinesterase inhibition) was observed following exposure via these routes in oral, dermal and inhalation toxicity studies.

$$\frac{1}{\frac{1}{MOE_{Oral}} + \frac{1}{MOE_{Dermal}} + \frac{1}{MOE_{Inhalation}}}$$

III. FQPA CONSIDERATIONS

1. Neurotoxicity Data

In an acute delayed neurotoxicity study, Malathion (93.6%) was administered by gavage to atropinized hens at 1007.5 mg/kg (1.3 x the oral LD50 of 775 mg/kg). A second dose (852.5 mg/kg) was given by gavage at study day 21. Mortality was extensive (only 14/60 hens survived the full study). Clinical signs of neurotoxicity, considered to be due to inhibition of cholinesterase activity, were observed for up to 6

days after dosing. No further clinical signs or gross or microscopic evidence of neuropathology was observed. (MRID No. 40939301)

In an acute neurotoxicity study, groups of Sprague-Dawley rats (27/sex/dose) received a single oral administration of Malathion (96.4%) in corn oil at doses of 0, 500, 1000, or 2000 mg/kg. For neurotoxicity, the NOEL was 1000 mg/kg and the LOEL was 2000 mg/kg/day based on decreased motor activity at peak effect time (day 1) and clinical signs (salivation, body staining, one death with tremor, labored breathing, stained fur, decreased defecation and urination). Plasma and erythrocyte cholinesterase were inhibited in both sexes at 2000 mg/kg on Day 7, a finding which was sustained, in females only on Day 15. Also, there was an equivocal inhibition of plasma cholinesterase for females at 500 and 1000 mg/kg, characterized by a poor dose response. No inhibition of brain cholinesterase activity was seen in either sex at any dose level. Equivocal neuropathological findings at 2000 mg/kg included axonal degeneration in the lumbar root and bilateral retinal rosette in one male, digestion chambers in the lumbar dorsal root fibers in one male and in the sciatic and tibial nerve in another male rat. The one rat with bilateral retinal rosette was observed was among but five males examined histopathologically in the high dose group, and that none were examined in lower lose groups. Digestion chambers and axonal degeneration of the sciatic nerve were also seen in one male control rat. (MRID No. 43146701).

In a subchronic neurotoxicity study, groups of Sprague-Dawley rats (25/sex/dose) were fed diets containing Malathion (96.4%) at 0, 50, 5000 or 20,000 ppm (0, 4, 352, or 1486 mg/kg/day in males and 0, 4, 395, or 1575 mg/kg/day in females, respectively). For systemic toxicity, the NOEL was 5000 ppm (352/395 mg/kg/day for M/F) and the LOEL was 20,000 ppm (1486/1575 mg/kg/day in M/F) based on decreased body weight and food consumption and on increased clinical signs (anogenital staining, and dried red material around the nose). For cholinesterase inhibition, the overall NOEL was 50 ppm (4 mg/kg/day) and the LOEL was 5000 ppm (352/395 mg/kg/day in M/F) based on inhibition of plasma and red blood cell cholinesterase in males and females and on Brain cholinesterase in females. There were no treatment-related effects on brain weight or neuropathology (MRID No. 43269501))

In a published study by Desi *et al.* (1976) Malathion (95%) was administered to female CFY rats at dietary doses of 38 and 75 mg/kg/day for 90 days. The authors reported that maze performance was affected during the first 21 days of the study and EEG and EMG recordings were affected after 90 days. Brain cholinesterase activity was inhibited, but clinical signs of cholinergic poisoning were not observed during the study.

In an *ad hoc* meeting of HED neurotoxicity experts convened to consider this study (and certain other studies on Malathion), the consensus of the meeting participants

was to perform a literature search on this finding and related findings for organophosphates in general and for Malathion in particular and if warranted by new information, consider requesting additional neurotoxicity studies on Malathion. The conclusins of this *ad hoc* meeting is attached to this Report.

2. Determination of Susceptibility

There is no indication of additional sensitivity to young rats or rabbits following pre- and/or postnatal exposure to Malathion in the developmental and reproductive toxicity studies.

(I) Developmental Toxicity:

In a prenatal developmental toxicity study, pregnant Sprague-Dawley rats (25/group) received oral administration of Malathion (94%) in corn oil at doses of 0, 200, 400, or 800 mg/kg/day during gestation days 6 through 15. For maternal toxicity, the NOEL was 400 mg/kg/day and the LOEL was 800 mg/kg/day based on decreased body weight gain, decreased food consumption and clinical signs of toxicity (urine-stained abdominal fur). No developmental toxicity was observed. For developmental toxicity, the NOEL was ≥800 mg/kg/day (HDT); a LOEL was not attained. Neither maternal nor fetal cholinesterase levels were measured in this study. (MRID No. 41160901)

In a prenatal developmental toxicity study, pregnant New Zealand White rabbits (20/group) received oral administration of Malathion (92.4%) in corn oil at doses of 0, 25, 50, or 100 mg/kg/day during gestation days 6 through 18. For maternal toxicity, the NOEL was 25 mg/kg/day and the LOEL was 50 mg/kg/day based on reduced mean body weight gains during treatment. For developmental toxicity, the NOEL was 25 mg/kg/day and the LOEL was 50 mg/kg/day based on a slightly increased incidence of mean resorption sites per dam. Neither maternal nor fetal cholinesterase levels were measured in this study. (MRID No. 40812001).

In a Range-Finding study, pregnant New Zealand white rabbits (5/group) received oral administration of Malathion (92.4%) in corn oil at doses of 0, 25, 50, 100, 200, or 400 mg/kg/day on Gestation Days (GD) 6-18. No mortalities or clinical signs were observed at 25, 50 or 100 mg/kg/day. At 200 mg/kg/day, 2 does died, 1 on GD 11 and another on GD 17. At 400 mg/kg/day, 4 does died, 1 on GD 7, 1 on GD 8 and 2 on GD 9. Cholinergic signs of toxicity seen at 200 and 400 mg/kg/day included tremors, decreased activity and salivation. External examinations of the fetuses did not indicate any gross abnormalities. For Maternal Toxicity, the NOEL was 100 mg/kg/day and the LOEL was 200 mg/kg/day based on mortality and clinical signs (MRID No. 00152569).

(ii) Reproductive Toxicity:

In a two-generation reproduction study, groups of Sprague-Dawley rats (25/sex/group) were fed diets containing Malathion (94.0%) at concentrations of 0, 550, 1700, 5000, or 7500 ppm (43, 131, 394, or 612 mg/kg/day in males and 51, 153, 451, or 703 mg/kg/day in females, respectively). For parental systemic toxicity, the NOEL was 5000 ppm (394/451 mg/kg/day in M/F) and the LOEL was 7500 ppm (612/703 mg/kg/day in M/F) based on decreased P generation body weights during gestation and lactation and decreased F1 pre-mating body weight. No effects on reproduction were observed. For offspring toxicity, the NOEL was 1700 ppm (131/153 mg/kg/day in M/F) and the LOEL was 5000 ppm (394/451 mg/kg/day in M/F), based on decreased F1a and F2b pup body weights during lactation. In the F1b and F2a litters, the pup weight decrements were observed at 7500 ppm (612/703 mg/kg/day). Although the DER describes this as a developmental NOEL/LOEL, the only treatment-related Day 0 body weight decrease in pups occurs at 7500 ppm in the F1b litters. In fact, pup body weight decrements were primarily observed at postnatal day 21. Neither adult nor offspring cholinesterase was measured (MRID No. 41583401).

Although the offspring NOEL (131 mg/kg/day in males and 153 mg/kg/day in females) was lower than the parental systemic NOEL (394 mg/kg/day in males and 451 mg/kg/day in females), the Committee determined that this was not a true indication of increased sensitivity of offspring because: (l) pup body weight decrements were primarily observed at postnatal day 21; (ii) during that period (i.e., later portion of lactation), young rats consume approximately twice the diet per unit body weight as an adult rat consumes (i.e., 1 ppm in the diet of a young rat is approximately 0.1 mg/kg/day whereas in older rats, this ppm level is equal to 0.05 mg/kg/day) and (iii) the estimation of the test substance intake in pre-weaning animals is likely to be more than double the adult intake because of the availability of the test material both via the milk (lactation) and food, particularly after the mid point of lactation.

(iii). Information from the Open Literature:

These summaries are provided to develop a comprehensive picture of Malathion toxicity. The data have not been reviewed in depth, and no statement is made regarding the accuracy or quality of the data or reports.

In day 1-3 chicken embryos, Malathion appears to produce multiple malformations of the wing-level and trunk/leg level spinal cord, eye, tailbud, and cardiovascular system, some of which result from aberrations in the neural fold, with from 125 µg to 4 mg Malathion (Wyttenbach and Thompson, 1985).

Neurotoxic esterase and delayed neuropathology studies in hens were judged not to demonstrate a potential for Malathion to cause delayed neurotoxicity. (Erich et al, 1995 and Jianmongkol et al, 1996)

Oral administration of Malathion at 0, 10 or 20 µg on gestation days 6, 9, or 12 to white mice did not result in developmental toxicity (Mufti and Safdar, 1991).

A study of Malathion exposure to sheep (20 mg/kg/day) on gestation months 3-5 resulted in ataxia, hind leg weakness and depression in the dams and abortion, still births, dystocia, placental retention, and low birth weight lambs. (Thatoo and Prasad, 1988).

A case study demonstrated delayed neurotoxicity in a suicide attempt exposure to Malathion (100 ml of 50% Malathion); findings may have been exacerbated by chronic alcoholism. (Komori et al, 1991).

A case study suggested a relationship between Malathion exposure (via head lice treatment shampoo during the 11th to 12th week of pregnancy) and a malformation of the nervous system development (amyoplasia-like condition) in an infant (Lindhout and Hageman, 1987).

An *in vitro* study of human fetal brain and liver suggested that "Malathion "altered the level of enzymes associated with glutathione cycle and antioxidase defense system", involving "alterations in glutathione status and extent of lipid peroxidation." The effect was greater in brain tissue than liver, and greater with earlier developmental stage, suggesting to the authors that there is a greater susceptibility of the human fetus to Malathion (Gupta et al, 1991).

Epidemiological surveys of pregnant women exposed to Malathion in Chile (Arevalo et al, 1987), Germany (Spielman, 1986), and the San Francisco area (Thomas et al, 198?; Grether, et al., 1987) suggested no adverse effects. A preliminary review of the study by Thomas and Green indicates that the San Francisco studies included groups of large sample sizes (7,450 and 22,465 births) which presumably should have resulted in statistically robust conclusions.

3. Recommendation for a Developmental Neurotoxicity Study

The Committee determined that, based on a weight-of-the-evidence review of the available data, a developmental neurotoxicity study with Malathion in rats is not required at this time. The following information was considered in arriving at this decision.

(I) Evidence that support requiring a developmental neurotoxicity study:

- Malathion is a neurotoxic organophosphorus pesticide. Administration to various species (human, rat, mouse, dog) causes inhibition of cholinesterase activity in various compartments.

- Some equivocal neuropathology was observed in the perfused tissues from the acute neurotoxicity study in rats.

- Minimal equivocal learning and memory effects were observed in the study in rats by Desi *et al.*

- A case study from the open literature indicated that delayed neuropathy resulted from a suicide attempt in an adult human male with chronic alcoholism. (This study was not supported, however, by other data in the literature or by the results of animal studies.)

(ii) Evidence that do not support asking for a developmental neurotoxicity study:

- No evidence of abnormalities in the development of the fetal nervous system, were observed in the prenatal developmental toxicity studies in either rats or rabbits, at maternally toxic oral doses up to 800 or 100 mg/kg/day, respectively.

- Neither brain weight nor histopathology (perfused or nonperfused) of the nervous system was affected in subchronic and chronic toxicity studies in several species, and in the neurotoxicity studies in rats.

- Available epidemiological data did not find adverse effects associated with exposure of pregnant human females to Malathion.

4. Determination of Uncertainty Factor:

The Committee determined that for Malathion, the **10 x factor** to account for enhanced sensitivity of infants and children (as required by FQPA) **should be removed.** This conclusion was based on the following factors.

(I) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

(ii) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.

(iii) The toxicology data base is complete and there are no data gaps.

IV. DATA GAPS

The toxicology data base is complete for Malathion; there are no data gaps.

V. OTHER ISSUES

A. Resolution of Issues Related to Neurotoxicity

The Committee determined that an *ad hoc* group should resolve three outstanding issues related to the neurotoxicological testing of Malathion. The three issues identified were:

1). The possibly greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of Malathion, and how this sex difference might affect the RfD for this chemical

2). Should the Agency require the Registrant to submit the microscopic slides (or photomicrographs) of retinal tissue from three rats in the acute and subchronic neurotoxicity studies on Malathion?

3) Should the Agency require the Registrant to perform and submit additional neurotoxicity studies to evaluate possible effects of Malathion on learning and/or behavior and/or other neurotoxicological parameters as exemplified in a literature article by Desi et al. (1976) in which maze performance (learning) and EEG and EMG recordings were reported as being affected in rats treated with Malathion?.

The conclusions of the *ad hoc* group meeting of November 13, 1997 are in Attachment 1.

B. Minority Reports

Three "Memorandums" from Brian Dementi, Toxicologist, to Clark Swentzel, Chairman, Hazard Identification Assessment Review Committee dated November 10, November 20, November 25, and one Memorandum from Brian Dementi, Toxicologist, to Jess Rowland, Executive Secretary, Hazard Identification Assessment Review Committee dated December 11,1997 are in Attachment 2.

VI SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected as well as Margins of Expsoures (MOE's) for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY	MOE
Acute Dietary	NOEL =50.0	Maternal toxicity	Range-Finding & Main Developmental toxicity studies - rabbits	100
Chronic Dietary	NOEL=4.0	Inhibition of plasma cholinesterase activity	Combined/Chronic Toxicity Carcinogenicity - Rat	UF= 100
Short-Term (Dermal)	NOEL =50.0	Inhibition of plasma, RBC and brain cholinesterase activity	21-Day Dermal - Rabbit	100
Intermediate-Term (Dermal)	NOEL=50.0	Inhibition of plasma, RBC and brain cholinesterase activity	21-Day Dermal - Rabbit	100
Long-Term (Dermal)	NOEL=4.0	Inhibition of plasma cholinesterase activity	Combined/ Chronic Toxicity - Rat	100
Short-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBCcholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	100
Intermediate-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBCcholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	300
Long-Term (Inhalation)	LOEL= 0.1 mg/L	Inhibition of plasma, and RBCcholinesterase activity & histopathology in respiratory epithelium	90-Day Inhalation Toxicity	300

ATTACHMENT 1 FOR THE 12/17/97 HIARC REPORT

Malathion: Report on the *ad hoc* Neurotoxicity Subgroup Meeting of November 13, 1997

December 3, 1997

MEMORANDUM

SUBJECT: Malathion: Report on the ad hoc Neurotoxicity Subgroup Meeting of November 13, 1997

DP Barcode D240967 Pesticide Chemical No. 057701
(Subbean to D238907) Tox Chemical No. 535
Case 818961
Submission S529758

FROM: Edwin R. Budd, Toxicologist
Registration Action Branch 2
Health Effects Division (7509C)

TO: Clark Swentzel, Chairman
Hazard ID SARC
Health Effects Division (7509C)

THRU: Richard Loranger, Branch Senior Scientist
Registration Action Branch 2
Health Effects Division (7509C)

INTRODUCTION

At the request of the Hazard ID SARC, which met on November 6, 1997 to conduct a toxicological assessment on malathion, an ad hoc neurotoxicity subgroup was formed to consider and resolve three outstanding issues related to the neurotoxicological testing of this chemical. The seven persons comprising this subgroup were nominated by Clark Swentzel and Mike Ioannou on November 6, 1997 and were the following: Clark Swentzel, William Sette, Kathleen Raffaele, Robert Fricke, Virginia Dobozy, Brian Dementi, and Edwin Budd (all staff members of HED). The subgroup met on November 13, 1997 from 1:00 to 3:15 PM. This report presents the decisions of the subgroup and will be appended to the final Hazard ID SARC report.

ISSUE #1--The possibly greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of malathion, and how this sex difference might affect the RfD for this chemical.

Discussion: On November 6, 1997, the Hazard ID SARC decided to base the RfD for malathion on the results of the 2-year combined chronic feeding/carcinogenicity study on rats (MRID 43942901). For the purpose of setting the RfD, the SARC considered the NOEL for inhibition of cholinesterase activity in this study to be 50 ppm in the diet (equivalent to 4 mg/kg/day in males and 5 mg/kg/day in females). A 32-56 day oral study in humans (males only)(Moeller and Rider, 1962) with a NOEL for inhibition of cholinesterase activity of 0.23 mg/kg/day was also discussed by the Hazard ID SARC and considered to be supportive of the RfD.

Subsequent to the November 6, 1978 meeting and during the neurotoxicity subgroup meeting on November 13, 1997, the issue was raised as to whether it would have been more appropriate to base the RfD for malathion on the results of the human study, rather than on the rat study. After considerable discussion, Clark Swentzel, in the capacity of chairman of the Hazard ID SARC, agreed to discuss this matter with selected members of the SARC to determine whether or not the full SARC might or might not be asked to readdress the choice of studies on which the RfD for malathion is based.

Regarding the possibly greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of malathion, the results of cholinesterase determinations in numerous studies on malathion were discussed and it was agreed that females do indeed appear to be more sensitive than males. There was not full agreement, however, on the relative degree of increased sensitivity of females compared to males. Also, there was not full agreement on whether or not a modifying factor should be applied to the RfD for malathion if the human study (in which only males were tested) were eventually selected to be the study on which the RfD for malathion were based.

Recommendation: The consensus of the neurotoxicity subgroup was that if the human study were eventually chosen as the basis for the RfD, it would not be appropriate to apply an additional modifying factor to the RfD to account for the increased sensitivity of females as compared to males. The rationale for this recommendation was that although a sex difference in sensitivity apparently does exist, the difference appears to be small. In many (but not all) studies, the sex difference did not result in different cholinesterase NOELs for males and females, but rather in different degrees of cholinesterase inhibition for males and females at a given dose level. It was pointed out that NOELs, rather than degrees of effect at a given dose level, are used in HED to determine RfDs and as the basis for various other risk assessment calculations. It was also pointed out that this same issue (possibly greater sensitivity of one sex) had arisen several times in the past with

respect to setting the RfD for other chemicals and that as a general policy it had previously been decided that additional modifying factors based on possible sex differences ordinarily would not be applied to RfDs.

The neurotoxicity subgroup also agreed that if the 2-year combined chronic feeding/carcinogencity study in rats were retained by the Hazard ID SARC as the basis for the RfD, the question of whether or not to apply an additional modifying factor based on sex to the RfD would be "moot" since 50 ppm (equivalent to 4 mg/kg/day in males and 5 mg/kg/day in females) was the cholinesterase NOEL for both males and females in the study.

ISSUE #2--Should EPA require the registrant to submit the microscopic slides (or photomicrographs) of retinal tissue from three rats in the acute and subchronic neurotoxicity studies on malathion?

Discussion: In the draft DER for the acute neurotoxicity study in rats (MRID 43146701), it was observed that 1/5 high dose group male rats had a bilateral retinal "rosette". Since concerns had arisen in recent years regarding the possibility that exposure to malathion might affect the visual system of humans and/or experimental animals, and since treatment-related lesions of the visual system had been observed in studies with certain other organophosphate pesticides, the occurrence of the bilateral retinal "rosette" in this high dose animal was considered by the reviewer to be a potentially serious effect of the test material and to warrant full investigation into the pathology and possible cause of the lesion in this animal. Further, the lesion was most likely a very rare event in rats. Toward this end, several pathologists were contacted regarding the potential seriousness of this lesion. These pathologists included Dr. Lucas Brennecke (EPA consulting pathologist), Dr. Robert Dahlgren (the study pathologist) and Dr. C. B. Clifford (Charles River pathologist). In addition, in the past, considerable discussion of this matter among several HED staff members also occurred, but all without resolution of the question of whether or not to ask the registrant to provide the microscopic slides of the retina of this rat to EPA for further examination--together with the slides of the retina of a control rat in the subchronic neurotoxicity study (MRID 43268501) which showed a unilateral retinal "rosette" and the slides of the retina from a randomly selected control rat from the acute study. Since the term "rosette" lacks histopathological preciseness, the slides of the retina of the control rat were required to determine if the lesion in this animal was indeed the same or was different than that in the high dose animal. Prior to the neurotoxicity subgroup meeting, additional information on retinal rosettes derived from a National Library of Medicine literature search was provided by Virginia Dobozy. The neurotoxicity subgroup discussed all the available information and data.

Recommendation: The consensus of the neurotoxicity subgroup was that, based on the presently available information, EPA should not ask for the microscopic slides of the retinas of these three rats at this time. The rationale for this recommendation

included a weight-of the-evidence consideration of the following:

The lesion of concern (bilateral retinal rosette) occurred in only one high dose male rat in the acute neurotoxicity study.

A unilateral retinal rosette was also tentatively observed in one control male rat in the subchronic neurotoxicity study.

Drs. Brennecke and Dahlgren both concluded the retinal rosette in the high dose male rat was not of toxicological significance and was not due to treatment with malathion.

Dr. Dahlgren considered the cause to be a "developmental deficit which occurs at the time of retinal maturation".

The neurotoxicity subgroup also concluded that retinal rosettes in rats are most likely the result of abnormal proliferation and differentiation of developing retinal cells during neonatal life (i.e. during the first approximately 32 days after birth) and ordinarily are not likely to develop in mature animals as a result of treatment with xenobiotics.

In a reference book available to the subgroup (Ophthalmic Pathology of Animals, Saunders and Rubin, 1975), it was stated that "[Retinal] rosettes occur spontaneously in certain strains of inbred rats and in beagle and collie dogs."

ISSUE #3--Should EPA require the registrant to perform and submit additional neurotoxicity studies to evaluate possible effects of malathion on learning and/or behavior and/or other neurological parameters as exemplified in a literature article by Desi et al. (1976) in which maze performance (learning) and EEG and EMG recordings were reported as being affected in rats treated with malathion?

Discussion: In the subchronic neurotoxicity study in rats (MRID 43269501), a guideline study that included a "functional observational battery" (FOB) and motor activity measurements, treatment-related effects on these two parameters were not observed at the highest dose level tested--20000 ppm (equivalent to 1486 mg/kg/day in males and 1575 mg/kg/day in females). However, in a non-guideline subchronic neurotoxicity study in female rats (reported by Desi et al., 1976), which employed dose levels of 0, 38 and 75 mg/kg/day, malathion was reported to affect maze performance (learning/memory) during the first 21 days of the study (increased errors and increased running time) and to affect EEG and EMG recordings after 90 days. At the dose levels tested in the Desi et al. study, brain cholinesterase activity was inhibited about 20% at 21 days, but clinical signs of cholinergic poisoning were not observed. Therefore, learning/memory deficits and changes in EEG and EMG

recordings were reported in the absence of cholinergic clinical signs (i.e. at subclinical doses). Since the guideline subchronic neurotoxicity study (MRI(D 43269501) did not assess either learning/memory or EEG or EMG effects, it was recommended in the draft DER that the registrant be required to perform and submit additional neurotoxicity studies on malathion to evaluate possible effects on learning/behavior and EEG and EMG changes. A schedule-controlled operant behavior study (guideline 85-5) was suggested as a possibility. The neurotoxicity subgroup discussed the general subject of learning/behavior studies and also considered specific information pertinent to the Desi et al. study. In addition, a memorandum from R.C. MacPhail (Chief, Neurobehavorial Toxicology Branch/HERL/EPA) to John Doherty (HED) and Brian Dementi (HED), dated May 4, 1995, was available which commented on the Desi et al. study and on the potential regulatory usefulness of further neurotoxicity testing of malathion as recommended in the draft DER.

Recommendation: The consensus of the neurotoxicity subgroup was that, based on the presently available information, EPA should not ask for additional neurotoxicity studies on malathion at this time. It was recognized, however, that such studies might possibly be requested at some time in the future if there were sufficient justification for doing so. Toward this end, the subgroup suggested it would be appropriate to perform a literature search on 1) learning/ behavior effects of organophosphates in general, and 2) available information on malathion in particular. After the literature search was completed and if warranted by new information, the question of additional neurotoxicity testing for malathion might be reconsidered.

cc: Brian Dementi
William Sette
Kathleen Raffaele
Robert Fricke
Virginia Dobozy
Mike Ioannou
Diana Locke
Jess Rowland

ATTACHMENT -2 FOR THE 12/17/97 HIARC REPORT

Memorandum -1: From Brian Dementi to Clark Swentzel, November 10, 1997.

Memoradum: From Brian Dementi to Clark Swentzel, November 20, 1997

Memorandum: Brian Dementi to Clark Swentzel, November 25, 1997

Memorandum: From Brian Dementi to Jess Rowland, December 17, 1997

Clark Swentzel, Chairman

November 10, 1997

HazardID SARC
Health Effects Division

As a follow-up to the November 6, 1997 HazardID SARC on malathion, I am compelled to express in writing my disagreement with certain very important decisions rendered at that meeting. One such issue concerns the apparent decision of the Committee to shift the basis of the RfD for malathion from the NOEL in the human study (Moeller and Rider, 1962), which has served in this capacity for years, to the NOEL for cholinesterase inhibition in the 1996 F344 rat chronic toxicity/carcinogenicity study. The problems I have with this decision are developed as follows. Firstly, the decision was too precipitous. By this I mean that since this is such a critical end point for this pesticide, it should have been presented as an issue or topic well before the meeting to allow people to be better prepared for discussion. I view this as a problem inherent in the process in dealing with a chemical having an extensive scientific record. Accordingly, there must be opportunity for offering further arguments supportable by additional information.

To the extent that Moeller and Rider incorporates a valid assessment of the LOEL/NOEL for cholinesterase inhibition in human subjects, being based as it is on **both** plasma and erythrocyte cholinesterases, evidence suggests humans are at least 10-fold more sensitive than F344 rats for erythrocyte cholinesterase inhibition and even more sensitive with respect to the plasma enzyme. To explain this difference, someone at the meeting suggested that 1962 vintage malathion was of questionable purity and that impurities could explain the differences with respect to the 1996 product. However, it was not indicated that humans have historically been more sensitive, i.e. were more sensitive than rat as compared on the basis of earlier products and likely remain so as compared to the more recent Cheminova product. Critical to the sensitivity of organisms to malathion in the cholinergic sense is the presence and level in such organisms of carboxylesterase activity, an enzyme(s) which, via catalysis of hydrolysis of one carboxyethyl group on malathion (actually malaoxon as the cholinesterase inhibiting entity), compromises its cholinesterase inhibitory capabilities. As I indicated at the meeting, insects lack carboxylesterase activity, which is thought to explain the remarkable selective efficacy of malathion as an insecticide. Similarly, to the extent that mammals incorporate differential levels of carboxylesterase activity they are variably sensitive to the agent in the cholinergic sense. Published works show that while carboxylesterase activity is located in the plasma and liver of the rat, in humans the enzyme is found in liver but not plasma. (Exhibit 1) The greater sensitivity of humans as demonstrated in Moeller and Rider may have its explanation in differing carboxylesterase activity in man versus rat. However, whatever the explanation, the fact remains that Moeller and Rider demonstrates the greater sensitivity of humans as compared historically using malathion of existing purity at the time and would likely prove so today if compared using the recent Cheminova product. I present these views as a way of dismissing any notions that Moeller and Rider has any fundamental flaw, if it can be accepted that malathion used in that study was at least as pure as 1962 vintage technical malathion, though purity of malathion used in

the study was not provided. If it were a more highly purified product, then to the extent that such culprit cholinesterase inhibiting impurities as malaoxon and isomalathion were reduced, the concern about relative human sensitivity would be to that extent more enhanced.

In view of these considerations, greater scrutiny of the rat cholinesterase data than was had at the November 6 meeting would be essential before a shift could be made from human to rat data as the basis for deriving an RfD. Along these lines I have the following to say. The Cheminova malathion technical product is said to be more pure than the former American Cyanamid product. Before the Committee accepts such claim, members should have in hand the Confidential Statement of Formulation for the respective products for direct comparison by the Committee. This is particularly important with respect to levels of cholinesterase inhibiting impurities. Cheminova has submitted data showing higher LD50 values for their product versus the American Cyanamide product, but LD50 may not be a good reflection of how products may compare at low levels of exposure based on cholinesterase data. LD50 values may be confounded by a host of adverse effects of the test material including cholinesterase inhibition brought on by trace impurities of cholinesterase inhibiting entities that do not require activation and thus become relatively more important at high doses of malathion where metabolic conversion of malathion to malaoxon becomes more saturated. Actually, I must confess to the committee that I very carefully compared the two product compositions awhile ago and there are reduced levels of malaoxon and isomalathion in the Cheminova product versus the American Cyanamid product, but I would question the relative effects of these these entities at low doses where metabolic conversion of malathion to malaoxon is less saturated.

In developing the protocol for the recently (1996) submitted malathion chronic/carcinogenicity study, the registrant was advised by our staff that 100 ppm, which the registrant was proposing as a low dose for the study, included principally in search of a NOEL for cholinesterase inhibition, would likely not be a NOEL for the blood borne cholinesterases. (Exhibit 2) It was explained that 100 ppm (lowest dose tested) was not a NOEL in the 1980 chronic/carcinogenicity study in the Sprague-Dawley rat, and likely would not be a NOEL in the new study. Nontheless, the registrant elected 100 ppm as the low dose for the new study, partly predicated on their view that their product is more pure than the American Cyanamide product empolyed in the earlier studies. As it developed, after 3 months on test, statistically significant erythrocyte cholinesterase inhibition was observed in females, prompting a reduction of the low dose to 50 ppm for rats of both sexes for the duration of the two year study in search of a NOEL. (Exhibit 3) I should note at this point that this finding corroborated the finding in the Sprague-Dawley rat performed seventeen years ago using tha American Cyanamid product. Subsequent to the three month time point, 50 ppm proved to be a NOEL for erythrocyte cholinesterase for both sexes. Firstly, what this says to me is that there is little if any improvement in the Cheminova product over that of the American Cyanamide product with respect to inhibition of erythrocyte cholinestyerase at low doses, particularly those critical to setting the RfD for malathion. Secondly, in the DER for the new chronic/carcinogenicity study in the rat, additional cholinesterase information is called for in view of the absence of a NOEL for cholinesterase inhibition among females at

the 3 month time point. It is alleged in the DER that given the ability of organisms to adapt somewhat to cholinesterase inhibitors (see, for example, the recovery of erythrocyte cholinesterase inhibition for females at 500 ppm at 6 months in that study, Exhibit 4), there is no assurance that the enzyme would not have been inhibited at 50 ppm during the first three months, i.e. during a very critical time frame for exposure to a pesticide.

This is also very important in view of the facts that, a) malathion has a very shallow dose response curve (in my judgement there is very little difference between 50 and 100 ppm for an agent that demonstrates such a shallow dose response curve ranging up to 6000-12000 ppm), b) the human study demonstrated greater sensitivity for uncertain reasons and c) the number of animals assayed for cholinesterase activity, 10/sex, does not accord sufficient statistical power to clearly identify a NOEL at low but meaningful levels of inhibition. I must maintain at this point that a definitive NOEL for cholinesterase inhibition be determined over at least a three month period using large numbers of rats at doses that embrace those employed in Moeller and Rider (.11-.34 mg/kg/day) overlapping those of the lower dose range of the rat chronic/carcinogenicity study, say up to 20 mg/kg/day. To the extent that this end point will be employed in establishing the RfD for malathion, I view it imperative that this data be gathered.

In summary I consider it inappropriate to change the basis of the RfD for malathion from the Moeller and Rider human study to the recently submitted chronic toxicity/carcinogenicity study in the F344 rat, particularly without a definitive NOEL for cholinesterase inhibition over the first three months of testing in ths case of the rat. Also, I recommend additional study to obtain a more definitive NOEL for cholinestarase inhibition at low doses in the rat

cc Jess Rowland

Brian Dementi
Toxicologist,
HED.

EXHIBIT 1

COMMENTS ON THE POTENTIAL ROLE OF ALIESTERASES
IN MALATHION TOXICOLOGICAL ASSESSMENTS

As reported in several sources, e.g. Dauterman (1971) there are various ca.rboxlesterases in the plasma and tissues of animals. Certain of these enzymes may play a significant role in the differential expressions of malathion toxicity. Dauterman cites references attesting to the presence of such enzymes as widely distributed in mammals and as having been found in the liver, kidney, serum, lung, spleen and ileum of the rat, mouse, guineapig and dog. "This hydrolysase is present in certain malathionresistant insects and it is reasonable to assume that resistance to malathion is at least partly due to carboxylase activity" (p. 139) Dauterman notes that the enzyme will hydrolyze only one of the two carbethoxyl groups on malathion.

Augustinsson (1959) presented research results and a good discussion on various types of carboxylesterases. Each type is actually a class or group of enzymes. The carboxylesterases might be defined as (1) aryl esterases - those which catalyze hydrolysis of aryl (aromatic) esters; (2) aliesterases - those w I hich catalyze hydrolysis of both aliphatic and aromatic esters and; (3) cholinesterases - those which catalyze hydrolysis of cholinesters. There can be overlap in enzyme spec t ificity.

The importance of aliesterases with respect to the toxicological profile for malathion might be explained as follows: unlike most organophosphates employed as pesticides, malathion has two carboxylester groups which in principle are vulnerable to hydroysis catalyzed by aliesterases. Once one of these ester 2 groups is hydrolyzed to yield a carboxylic acid substituent on the residual malathion molecule, the molecule looses its cholinesterase inhibiting capability and' hence, looses its cholinergic toxicity. The structure of malathion is as follows:

********* STRUCTURES NOT PRESENTED HERE.....SEE FILE COPY******

Reactions possibly catalyzed by aliesterases would yield, in principle, any of the following three molecules:

*******MOLECULES NOT PRESENTED HERE.....SEE FILE COPY******

As reported, only one carboxylester group is cleaved on the molecule, which yields compound B resulting from action at the alpha carboxylester group. -Apparently this is the preferred site for the enzyme and once cleavage occurs, the carboxyl group generated precludes further binding of the molecule to aliesterase (Dauterman, 1971, p. 142). Of course, the product molecule will not inhibit cholinesterase. This is further substantiated in the work of Wilkinson (1976): "It is probable that the selectivity of malathion is directly related to the presence or absence of carboxylesterases in. various species. Thus, carboxylesterase activity is found to be low or absent in several insect species susceptible to malathion (Kojima, 1961) and is usually high in malathion resistant strains or species (mot'oyama and Dauterman, 1974)." (p. 157)

Augustinsson (1959) examined the plasma of several species for all three types of esterases. Essentially, he found that while aliesterases are present in the plasma of many species, for example, rat, rabbit, horse, cat, guinea-pig, etc., this enzyme is absent from the plasma of the human, monkey and dog. Thus, to the extent that the enzyme is missing from human plasma, humans would lack this essential line of defense against cholinesterase inhibition by malathion, once oxidized to malaoxon. By contrast,' the rat, rabbit, guinea-pig, etc. possess 'this capability in the plasma to detoxify malathion (cholinergically) . This conclusion or rationale is further supported by the work of Main and Braid (1962) who demonstrated the essential absence of aliesterase activity in human serum, though finding it abundantly present in rat serum. These investigators were able to show that when serum aliesterase is inhibited in the rat using tri-o-tolylphosphate (TOTP) , the acute toxicity of malathion was remarkably enhanced. For example, the LD50 of secondary standard malathion as reported by these investigations for the rat is,.*1600* mg/kg. However, when administered one-hour post TOTP administration at doses inhibiting aliesterase activity, the malathion LD50 dropped to 35 mg/kg. Similarly, the LD50 of technical malathion dropped from 415 mg/kg to 7.5 mg/kg when TOTP was employed. The implication of this work is that with respect to anticholinesterase activity (and, hence, cholinergic effects) malathion toxicity may be greater in the human than in the rat. Indeed, according to Main and Braid, "The hydrolysis of malathion by aliesterase explains the vast difference between the toxicity of compounds, such as parathion and malathion.11 (p, 262). It should be noted that humans are not devoid of aliesterase activity. Human liver contains aliesterase activity, as does rat liver (Main and Braid, P. 257). Hence, according to Main and Braid: "It is difficult at this time to predict precisely the toxicity of malathion toward human beings on the basis of the detoxicating effect of aliesterase in the rat. The complete absence of aliesterase activity in human serum means that at lease one important barrier to malathion poisoning present in the rat is absent in humans.11(p. 262) (A related reference is that of Ecobichon and Comeau, 1973). It should also be emphasized that the dog probably contains no serum aliesterase, but, as is true in the case of man, dog liver contains aliesterase activity. In fact, Augustinsson (1959) notes that esterase electrophoretic patterns of the plasma of the dog resembled that of human plasma (p. 584). "Human, monkey, dog, swine and ruminant plasmata do not contain aliesterasell (p. 591) However, Augustinsson (pp. 584-85) appears equivocal as to whether there may be some aliesterase activity in dog plasma. The absence

of aliesterase in dog serum is further substantiated by the work of Murphy and DuBois (1957).

"The serum of mice and rats was capable of detoxifying malaoxon, but dog serum exhibited no activity" (p. 815). These authors also report a 4-fold difference in this activity of the liver of male rats with respect to that of female rats, male's being more active.

The latter comments offered here with respect to the dog are ,designed in part to help assess the suggestion of Dr. A. A. Sadun (letter to B. Dementi, May 25, 1990) in which he advocates the dog as a better surrogate than the rat for man in the event ocular testing is pursued. The absence of aliesterase activity from the dog serum and the similarity of dog to man with respect to aliesterase profiles would support use of the dog over the rat in such testing. This would be expected to be true more so if anticholinesterase activity is important in the etiology of any -effects. However, the ocular organohosphate phenomenon might not be entirely cholinergic in nature, and malathion remains an organophosphate even after a carboxyethyl group is hydrolyzed. Nevertheless,based on this line of reasoning, it does appear the dog would be preferred to the rat as the surrogate. The articles cited above are appended. There is much noteworthy information in these articles. A thorough up-to-date search of the literature followed by review would be desirable in order to more definitively characterize the role of aliesterases in malathion toxicity. For now, information on aliesterases developed from these articles must be viewed as helpful.

ADDITIONAL NOTES:

(1) The, absence of aliesterases in human plasma could serve to explain the lower cholinesterase NOEL in humans (Moeller and Rider, 1962) than in rats. Hence, the malathion RfD based upon Moeller and Rider assumes more significance in view of the aliesterase distinction between man and the rat, for instance.

(2) The prolonged duration of malathion in human serum allows more time for conversion to malaoxon.

(3) Aldridge (1953) reported investigations showing that E600-esterase (an enzyme which hydrolyzes E600, (diethyl-pnitrophenyl phosphate) at the phosphate center to yield p-nitrophenol) is the same as arylesterse, the enzyme known to hydrolyze such arylesters as p-nitrophenylacetate, p-nitrophenyl propionate and p-nitrophenylbutyrate. The article appears to say that aliestereses' will also hydrolyze the latter carboxylic-acid esters, but is mute as to whether aliesterases with hydrolyze E600. To the extent that E600 esterase (arylesterase) will hydrolyze E600, it is behaving as a phosphatase; however, the article makes clear that arylesterases are different enzymes from the phosphatases. The article does not address the issue of how broad the phosphates - e activity of the arylesterases may be. Apparently this class will not catalyze hydrolysis-of diethylphenylphosphate. Also, apparently aliesterases will hydrolyze p-nitrophenylactate, p-nitrophenylpropionate and p-nitrophenylbutyrate. It is doubtful that aliesterases will hydrolyze E600.

In any case, the author indicates the destinction between arylesterases and both aliesterases and phosphatases. This whole subject of hydrolysases in plasma and tissues of various species is a complex one, but of great interest. The literature discussed above represents but a partial effort. A thorough reviaew of the literature followed by a comprehensive written review would be desirable.

Brian Dementi, Ph.D., D.A.B.T,
Toxicology Branch I
Health Effects Division

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EXHIBIT 2

JELLINEK, SCHWARTZ, CONNOLLY & FRESHMAN, INC.

February 13, 1992

Ms. Joanne Edwards
Product Manager (74)
Special Review and Reregistration Division (H7508CO)
Reregistration Branch
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Dear Joanne:

This letter contains our minutes on what transpired at our meeting on malathion with a member of your staff and two HED toxicologists on December 10, 1991. We would appreciate your concurrence with these minutes and the conclusions we reached concerning testing of malathion and malaoxon in chronic toxicity/oncogenicity studies. At the end of this letter, you wiU find a signature line for concurrence by HED toxicologists. If they do not concur with statements made in this letter that would affect the conduct of these studies, please inform us at your earliest convenience because testing is to be initiated early in 1992.

Those in attendance at the meeting included Mr. Jon Weis (CheminQva Agro A/S (Cheminova,)] and Dr. Judith Hauswirth (JSCF) representing Cheminova and Dr. Brian Dementi, Mr. Larry Schnaubelt, and Dr. Hank Spencer representing EPA/OPP.

Mr. Weis opened the meeting with a brief discussion of the transfer of the malathion registration in the United States to Cheminova solely. The only malathion technical product to be sold in the United States will be made by Cheonova. American Cyanamid will no longer be producing malathion for sale in the United States. As of December 31, 1991, the malathion task force no longer exists.

T'he discussion then turned to issues related to the conduct of rat chronic toxicity/oncogenicity studies on malathion and malaoxon and a mouse oncogenicity study on malathion. It was agreed that the malathion mouse oncogenicity study would be conducted for 24 months instead of 18 months, which would have been in accordance with the conduct of the NCI mouse oncogenicity study on malathion as requested by the Agency. Drs,.Dementi and Spencer agreed to this because of the proposed performing laboratory's (International De-lopment Corporation) historical control data base on studies of this length; Research and Deve
however, they requested that they be informed if survival past 18 months becomes a problem in the malathion study.

The two high dose levels (8,000 and 16,000 ppm) for this study have been previously agreed upon with the Agency. Dr. Dementi questioned why the low dose level was raised from 35 ppm, as originally proposed by American Cyanamid, to 100 ppm. Dr. Hauswirth stated that this was an American Cyanamid decision, that we would check into the reasoning, and that we would inform EPA of the reason as soon as we could.

The dose levels (20,000, 10,000, 5,000, and 100 ppm) selected for the chronic toxicity/oncogenicity study on malathion in the rat were discussed. Dr. Hauswirth presented the rationale for selection of the two high dose levels. The highest dose selected, 20,000 ppm, is the limit dose for studies of this type. The next highest dose level, 10,000 ppm, represents one-half of this dose level and would serve as the high dose for the oncogenicity study if mortality was excessive at the limit dose. The dose level of 5,000 ppm would serve as onehalf of the top dose if mortality were excessive at 20,000 ppm. Dr. Dementi asked why 100 ppm was selected as the lowest dose level tested because in his opinion in past studies conducted on malathion 100 ppm was an effect level. Dr. Dementi stated he would prefer the lowest dose level to be 50 ppm. We agreed that we would look into this issue but informed EPA that the Cheminova technical malathion is less acutely toxic than the American Cyanamid technical. EPA was not aware of the differences in acute toxicity. Mr. Weis said that the comparative acute studies would be submitted officially to EPA and he showed EPA copies of the studies for discussion. It was suggested by Cheminova representatives that 50, 100, 10,000, and 20,000 ppm might be more appropriate dose levels for this study in light of Dr. Dementi's concerns about a NOEL for cholinesterase inhibition and the differences in toxicity between Cheminova's and American Cyanamid's technical products. EPA thought this M be more appropriate.

In addition, with regard to the rat chronic toxicity/oncogenicity study with malathio n, Dr. Hauswirth noted that EPA had suggested that a 90-day range finding study be conducted prior to the chronic testing. Dr. Hauswirth, suggested that a 28-day study be conducted, initially because of the already available toxicity information for mala n in the Sprague Dawley rat and because this would cut down on the time it would take to initiate the two-year study. Dr. Dementi stated that he wants the chronic studies started on malathion as soon as possible and that he is very concerned about how long it has taken to initiate the studies. EPA and Cheminova agreed that this was a good approach to take and would be sufficient provided adequate data were provided for dose selection from the 28-day study results. If not, it was agreed that a 90-day study would be initiated perhaoin conjunction with neurotoxicity testing (a requirement of the draft data-call-in on malathion)..

Dr. Dementi requested that we submit the methodology that will be used for cholinesterase activity determinations. Cheminova committed to providing this information and asked for a quick turnaround time on review. Dr. Dementi assured us that we would get a quick response and that the methodology should be submitted to Joanne Edwards. Drs. Dementi and Spencer also asked that we inform them when the studies have been initiated and that we provide annual progress reports.

EPA and Cheminova agreed that the dose levels for the malaoxon chronic toxicity/ oncogenicity study have been previously approved by EPA. Dr. Dementi noted that ultimately dose level selection was the responsibility of the registrant. Mr. Schnaubeit informed Cheminova that the ocular toxicity and neurotoxicity testing guidelines are to be discussed at an EPA-sponsored workshop sometime in January and are therefore subject to change.

The meeting ended with a commitment from Cheminova to initiate the studies on malathion and malaoxon as soon as possible.

Diane Allemang
JSCF & Co., Inc.
Authorized Representative of
Cheminova Agro A/S

cc: Brian Dementi
Hank Spencer

Concurrence:

Dr. Brian Dementi

Dr. Hank Spencer

EXHIBIT

3

STUDY TITLE

Overview Of The Subchronic and Chronic Toxicity of Malathion

442797-01 I

DATA REQUIREMENT

U.S. EPA Pesticide Assessment Guidelines, Subdivision F:
Toxicity Testing

AUTHOR

Jellinek, Schwartz & Connol,ly, Inc.
1525 Wilson Boulevard, Suite 600
Arlington, VA 22209-2411

STUDY COMPLETED ON

May 30, 1997

SPONSOR

Cheminova Agro A/S
DK-7620 Lemvig
Denmark

Table 5. Actual M-alathion Intake Based on Food Consumption

Dosage **Level (ppm)**	Mg/Kg/Day	
	Males	**Females**
100	4	5
500	29	35
6000	359	415
12,000	739	868

These dose levels were considered reasonable by the Health Effects Division (HED) (memorandum dated January 25, 1993, from Brian Dementi to Joanne. Edwards) based on the results of the 90-day study and a previously conducted 28-day study; however, The HED was concerned that I 00 ppm might not be a no-observed-effect level (NOEL) for cholinesterase inhibition.

In the definitive study, 90 Fischer 344 rats/sex/group were placed on test. Ten rats/sex/group were evaluate at 3, 6, and 12 months for clinical laboratory studies and cholinesterase activity, including brain. Necropsy was performed on 10 rats/sex/group at 3 and 6 months, and on 15/sex/group at 12 months. Electroretinograms (ERGS) and fundic photo evaluations were performed on select animals at various times during the study. The remaining 55 rat/sex/group remained on test until study termination at month 24.

The HED's concerns regarding a NOEL for cholinesterase inhibition were confirmed when, at the three-month time period, RBC cholinesterase inhibition was statistically decreased in female rats at all dose levels tested (Table 6). At this point in the study the low dose of I 00 ppm was dropeed to 50 ppm in both males and females. Six weeks after this reduction in dose level, RBC cholinesterase was evaluated in 10 animals/sex from the control and 50 ppm dosage groups. RBC cholinesterase levels in animals at 50 ppm were comparable to the control group. The NOEL for plasma cholinesterase inhibition was also 50 ppm, based on statistical reduction in females only at 12 and 24 months in the 500 ppm group. The NOEL for brain cholinesterase inhibition was 500 ppm.

EXHIBIT 4

Huntingdon
Life Sciences

Data Recuirement

Test Guideline 83-5

STUDY NO. 90-3641

A 24-MONTH ORAL TOXICITY/QNCOGENICITY STUDY OF MALATHION IN THE RAT VIA DIETARY ADMINISTRATION

Final Report

VOLUME I OF XIV

Author: Ira W. Daly, Ph.D., D.A.B.T.

Performed by:Huntingdon Life Sciences Mettlers Road
P.O. Box 2360
East Millstone, New Jersey 08873

Sponsor: Cheminova Agro A/S
P.O. Box 9, OK-7620
Lemvig, Denmark

Date:27 February 1996

Page I of,5666

H. Clinical Laboratory Studies (Cont)

2. Cholinesterase Evaluations (cont)

Mean Choinesterase Values														
Group	Dose Level (ppm)	Plasma (IU/mL)				Erythrocyte (IU/mL)					Brain (IU/mg)			
Month		3	6	12	T	3	6A	6B	12	T	3	6	12	T
MALES														
I	0	0.623	0.633	0.737	1.869	0.90	0.75	1.03	1.43	1.16	10.77	10.02	9.93	10.76
II	50[a]	0.609	0.621	0.698	1.615	1.17**	0.72	1.16	1.43	1.11	10.82	9.85	9.83	10.59
III	500	0.631	0.591	0.736	1.327**	1.01	-	0.97	1.33	0.96	10.71	9.80	9.69	10.44
IV	6000	0.456**	0.481**	0.615*	0.675**	0.47**	-	0.59**	0.78**	0.66**	9.43**	8.85**	8.85**	7.41**
V	12000	0.292**	0.299**	0.419**	c	0.47**	-	0.44**	0.60**	c	9.10**	8.16**	8.45**	c
FEMALES														
I	0	2.928	3.330	3.319	3.495	1.46	0.99	1.37	1.50	1.35	10.75	9.99	9.89	10.73
II	50[a]	2.868	3.327	3.581	3.475	1.10**	0.85	1.38	1.52	1.34	10.78	9.68	10.09	10.69
III	500	2.760	3.305	3.408	2.879	1.02**	-	1.37	1.29*	0.99**	10.41	9.62	9.75	10.58
IV	6000	1.449**	1.777**	2.053**	1.374**	0.62**	-	0.77**	0.83**	0.76**	9.12**	8.27**	8.70*	8.78**
V	12000	0.566**	0.654**	1.004**	0.389**	0.49**	-	0.62**	0.74**	0.65**	6.72**	5.05**	7.17**	3.52**

[a]The low-dose level was reduced from 100 to 50 ppm following the Month 3 cholinesterase evaluation interval.

[b]Month 6A represents erythrocyte cholinesterase determinations performed o n 6/7/93 and 6/1193 (Week 23) 6 weeks after the reduction in dose level from 100 to 50 ppm. Month 6B represents the scheduled 6 month cholinesterase evaluations/sacrifice.

[c]Values were not available at the 24 month (terminal) sacrifice for the males at the 1200mppm level due to the lack of any survivors in the males at this dose level.

T = Termination, * = Statistically Significant $p \leq 0.05$, ** = Statistically Significant $p \leq 0.01$

Clark Swentzel, Chairman
Hazard ID SARC
Health Effects Division

November 20, 1997

Re: Ad Hoc Committee Meeting of November 13, 1997 on Malathion Issues

As a matter of the record, regarding the referenced meeting, this is to advise you that in spite of the good effort on your part to see that a fair and reasonable meeting was held, and I thought you did well, I do not consider the outcome satisfactory. The decisions made were very inadequate and not in the interest of the public health, as they compromise full pursuit of the understanding of the toxicology profile on this important and extensively used pesticide. No stone should be left unturned, given the enormity of human exposure to this cholinesterase inhibiting organophosphate. I shall comment on the topics that were the subject of the meeting in the order in which they were taken.

Retinal Anomaly in Acute Neurotoxicity Study on Malathion (MRID 43146701

I have presented fully my views on this subject in written documents, which were available to the Committee, and will not restate these views at this writing. The fact remains that the Acute Neurotoxicity Guidelines (81-8) call for sequential histopathologic evaluations of specific tissues in lower dose groups when histopathologic findings are noted in the high dose group animals. It would appear to me that this requirement should be met in this Guideline even if but one lesion is observed in a particular tissue of the high dose group given the small number of animals (5/sex) in a dose group. This was not done in the study in question after the one bilateral retinal rosette was noted in a high dose male group. Now it is not a source of happiness to me to be perceived as one who over-assesses a study, and this is why I feel very awkward in defending this position. If the one incident standing alone had been identified among fifty or more animals in a group, surely I would not have pursued the matter, but in this case given the rarity of the lesion in historical data bases and the uncertainty as to the lesions microscopic anatomic features (retinal rosette is not an anatomic term and on the face of it, the term could be used to apply to any of a variety of underlying morphologic changes), I felt that as a matter of the record, our pathologist should provide anatomic characterization. Also, there was somewhat greater incumbency to require this assessment since it involved the retina, in view of the prevailing concerns over possible retinal effects of organophosphates in general and of malathion in particular. While I did not say so at the November 13 meeting, it is essentially self-evident that the assesment of the requested slides could be instrumental in determining whether to insist upon examinations of lower dose groups as mandated in the Guidelines. For example, this might be contingent upon whether the bilateral retinal rosette of the high dose male in the acute study is morphologically or anatomically the same as that of the unilateral rosette of a control rat in the subchronic neurotoxicity study.

Lastly, I believe the relatively minor decision to ask for a couple slides should be entirely within the perview of the reviewer, given what may be his peculiar perspectives on the subject, without having it go before a committee for approval. As I said, for the record, this issue remains unresolved if the slides in question are not submitted.

Relative Sensitivity of Females Versus Males to Cholinesterase Inhibition by Malathion

I presented to the Committee several comparisons of the level of cholinesterase inhibition for males and females from our Guideline and dose range-finding studies on malathion and malaoxon. Although the magnitude of differences between the sexes is variable across studies, there is more than adequate evidence to establish a greater sensitivity for females. The ad hoc Committee did agree that sex-related differences are

manifest, but did not concur with the proposition that differences may merit a correction factor to be applied to male (human) data used as the basis for the RfD. It should be noted at this point that the RfD for malathion, 0.02 mg/kg/day, which ostensibly protects the entire human population - men, women, boys and girls of all ages- employs a mere ten-fold safety factor as applied to experimental data obtained on humans (men only). In the absence of such data for women and youths, in my judgement a larger safety factor than ten should be employed, particularly in the face of evidence that females are more sensitive to malathion than males as assessed in laboratory animal studies, and where studies of organophosphates in general suggest young individuals to be more sensitive. According to the 1988 malathion registration standard: "The Theoretical Maximum Residue Contribution (TMRC) for the U.S. population average is 0.1014 mg/kg/day, occupying 505% of the PADI. For children 1 to 6 years of age, the TMRC occupies 1133% of the PADI. The TMRC is based upon current tolerance levels and an assumption that 100% of the sites are treated. Actual dietary exposure may be much lower." (p.32) The point is that a much higher percentage of the PADI is consumed, or was so in 1988, than is to be desired, which places an enhanced scrutiny upon the reliability of the RfD in protecting real people.

Unfortunately I did not have the time before the meeting to provide study by study estimates of such correction factors, but am certain that a legitimate correction factor, whatever it is, would be of such magnitude that it should not be ignored, especially in view of the small safety factor used for the existing RfD. Additional study in animals may be necessary to properly identify the correction factor. Realizing that a sex-related differential sensitivity exists, unacceptable in my opinion is the Committee's out of hand rejection of the argument that a meaningful ratio exists without first obtaining some numerical estimates of that ratio of sensitivity from the data currently in hand. Indeed, I had anticipitated that an outcome of the meeting would be a Committee recommendation that such estimates be computed for subsequent consideration.

Testing for Effects on Learning/Memory

Again, available to the Committee were various documents presenting arguments pro and con that findings with malathion on learning/memory at very low doses in a published work, Desi et al. (1976), are of sufficient validity and concern to require Guideline testing of malathion for these effects. In addition to explaining to the Committee that the published work shows that malathion at doses of 38-75 mg/kg/day in a subchronic study elicited effects on learning/memory, EEG and EMG, as contrasted with no neurotoxic (motor activity, FOB parameters) effects in the Guideline subchronic neurotoxicity study at doses up to 1575 mg/kg/day, I had recommended that a Guideline test of learning/memory be required for malathion. The Committee rejected this recommendation on the grounds that Desi et al (1976) is not a reliable study. This criticism of the study was maintained in spite of many findings in the study that affirm its veracity. Of these I mentioned the facts that the stated purpose of the authors was to assess the effects of malathion at subclinical levels on sensitive neurotoxicity parameters including learning/memory; 95% malathion (American Cyanamid) was used; the authors affirmed the absence of clinical signs which was consistent with the low but meaningful level of cholinesterase inhibition; cholinesterase activity was remarkably well evaluated in the study, including assessments of plasma, erythrocytes and brain regions, where the findings were consistent with those of the Guideline subchronic neurotoxicity study (which in turn enhances the credibility of the published work), and adverse effects of malathion on kidney tissue in in vitro kidney tissue cultures being somewhat consistent with or supported by chronic nephropathy as the cause of increased mortality (100% and 74% in the high and penultimate doses, respectively) in the 1996 chronic toxicity/carcinogenicity study in the F344 rat. Furthermore, the authors of the study affirm in the text a real effect of malathion on learning and memory as assessed in their study.

The Committee members were mute with respect to acknowledging any of these facts as supporting evidence of the work by Desi et al, but persisted in criticizing the study on the grounds that the effects on

learning/memory in terms of errors made by rats in maze studies were small, not dose related between 38 and 75 mg/kg/day; that statistics were ill defined and that it would be surprising for malathion to exert such an effect at such low dose levels. I endeavored to explain that findings were in fact not small in terms of differences in errors made in dosed groups versus controls. I also offered my opinion that 38 and 75 mg/kg/day, when compared on the shallow dose response for malathion are actually not very different, and that brain cholinesterase inhibition was 20% in the two groups at 21 days, the time at which learning/memory was affected. These two observations would point to similar responses on tests of learning/memory, and thus the absence of a dose response as noted. I also explained from an earlier work by Desi et al, which the authors cited as background for methodology, that bar graphs in that study were said to be standard deviations, which if true in the 1976 study would mean that differences between controls and dosed groups on errors made in the learning /memory test would be statistically significant. In spite of these findings, plus the EEG and EMG data affirming a neurological effect of the test material at these dose levels, and in view of the fact that the Guideline subchronic neurotoxicity study was not designed to assess learning/memory, EEG or EMG effects that could refute the findings in Desi et al, the Committee categorically rejected the Desi study as of any relevance. In fact, I recall saying to the group, "It's as if Desi does not exist?", whereupon I was responded to in the affirmative. In my judgement, this qualifies as an authoritarian rejection of data the Committee failed to refute. I maintain that Desi et al (1976) in spite of its deficiencies is of sufficient quality that it conclusions, particularly with respect to the effects of malathion on learning/memory, mandate verification through proper Guideline testing procedures, which are available. As to the question of the "small" effect on errors made by rats in the learning and memory aspect of Desi, et al, one might ask, what is small? Imagine a high school student taking his algebra exam, on which his grade would be say 97, other things being equal, but under the influence of a xenobiotic he was exposed to, his score turned out to be 92 due to a few additional errors he made. Now a 92 (B) is a very good grade, but not quite as good as the grade he deserved 97 (A). One might say this is a small difference, but who would argue that is to be ignored?

I have concerns about the legitimacy of the opportunity presented to me to go before an unbiased ad hoc committee. I had reservations before the November 13 meeting that I should even pursue the matter. This concern was born out by the following episode that occurred at the meeting. As you will recall during the meeting, at the precise moment that we completed our deliberations on the second topic, one Committee member, arriving late, voted on the issue. In fact, as I recall, you commented at the time that so and so is voting even though she was not present during the discussion. From my perspective, her vote was more than improper in that it conveyed the impression, whether rightly or wrongly interpreted, that the Committee's conclusions were foreordained, and that my opportunity to be heard at this meeting was a mere formality. When I came to item three, my presentation was compromised in the psychological or motivational sense, given what had previously taken place. I could see "The handwriting on the wall" and thus the futility in proceeding further on what was really the most important of the three issues.

In my view, minds had been made up, and I felt nothing I said would matter before this Committee. Indeed, I came preciously close to calling off any further discussion, but felt that would be of no avail either, as people might then say "well, you had your chance", as if this were some kind of real and legitimate peer review. I am convinced it was so in name only. The bottom line to all this is that another forum for peer review of these issues is required, bearing in mind the importance of this subject to the public health. People composing a true peer review committee should be experts in the field, but at the same time should not have personal vested interest in HED.

Brian Dementi
Toxicologist, HED

cc Jess Rowland

Clark Swentzel, Chairman
Hazard ID Committee

November 25, 1997

RE: Malathion RfD

It is my intent here to comment further on certain issues before the Hazard ID SARC of November 6 and the Ad Hoc Committee meeting of November 13, 1997, with particular reference to the RfD for malathion.

In my memorandum to you of November 10, I endeavored to explain why the cholinesterase data in the recent chronic toxicity/carcinogenicity study of malathion is inadequate to define a NOEL for female F344 rats. As a remedy, I recommended a definitive three month assessment of cholinesterase inhibition in the rat. In my judgement, until such data are available, a gap exists with respect to the identification of a NOEL for the first three months of exposure to malathion, and, hence, proper data do not exist in this study upon which to poise an RfD. This being true, and to the extent that the Moeller and Rider (1962) study, performed in humans, may continue to be used as the basis for the RfD until proper rat data are obtained, the following comments are relevant.

At the Ad Hoc Committee meeting, when discussing the topic of greater sensitivity of females to cholinesterase inhibition by malathion, I expressed the view that for studies wherein cholinesterase inhibition was obtained in but one sex, as is true in Moeller and Rider where only male volunteers were tested, that a greater than the normal uncertainty factor (UF) of 10 should be applied. As I recall, this was not affirmed by any one at the meeting. I suspect no one felt sufficiently certain to render a definite opinion. In any case, I believe this is a question requiring an answer. I do not have the time to search the records, but I believe the answer should be readily available in the minutes of past RfD meetings, and should be a well recognized operating principle for the RfD Committee. I have just by chance reviewed the 1997 Registration Eligebility Document (RED) toxicology chapter for carbofuran, and I find in the case of the RfD that the Agency applied a UF of 100 to the NOEL for cholinesterase inhibition in male volunteers. Quoting from that RED chapter: "An uncertainty factor (UF) of 10 was applied to account for intra-species variability. An additional UF of 10 was applied to account for study deficiencies (use of limited number of subjects, few subjects/dose and **use of males only** (emphasis added)". Please be aware that Moeller and Rider, in addition to being a study in males only, has its inadequacies also (e.g., limited number of subjects, purity of the test material not provided, interpretation of low and mid dose effects somewhat confounded by co-administration of EPN).

In my memorandum to you of November 20, I quoted from the malathion registration standard, passages revealing how high the TMRC is (or was in 1988) when based on the RfD of 0.02 mg/kg/day, derived from Moeller and Rider with a UF of only 10. The Committee should be aware that at an earlier time point, a UF of 100 had been applied to Moeller and Rider, at which time the RfD was thus 0.002 mg/kg/day. Also at that time the TMRC was about 5000% of the PADI. At some point in time, and I don't have the details, I would estimate around 1987-90, the UF was reduced from 100 to 10, for reasons unknown to me.

I recommend that your Committee seek the historical record on the setting of the RfD for malathion, and make your own independent assessment of its reasonableness, as this is the moment in time for reconciling the RfD with the facts at hand. On the face of it, if a UF of 100 is appropriate for carbofuran for the reasons given, an explanation should be forth coming for the use of only 10 in the case of malathion. Please understand I am not saying a satisfactory explanation does not exist, but let us see it. I must maintain the view that when a UF of only 10 is employed, it is imperative that the study in question incorporate data on both sexes.

In summary, in my view proper data on cholinesterase inhibition in rats are not available at this moment to justify replacing the Moeller and Rider human study as the basis for the RfD for malathion. Furthermore, in the absence of cholinesterase data on women, the UF as applied to the Moeller and Rider human (men only) data should be revised upward from the 10 which is currently employed.

Brian Dementi, Ph.D.
Toxicologist/HED

cc Jess Rowland
George Ghali

Jess Rowland, Secretary
Hazard ID Committee

December 17, 1997

Comments on December 4, 1997 draft report of malathion Hazard ID Committee meeting of November 6, 1997. The following is the best I am able to produce given the constraints of time and the complexity of the subject.

Comments on the various endpoints are presented as follows in the order in which they appear in the draft report.

I Introduction (p. 1) O.K.

11 Hazard Identification

A. Acute Oral (one-day): For this endpoint, the Committee concluded that the 50 mg/kg/day dose is appropriate for acute dietary risk assessment. This endpoint is based upon decreased maternal body weight gain in the malathion developmental toxicity study in the rabbit (MRID 152569). In support of this, the draft Hazid ID Committee Report (HIDR) cites the DER for the rabbit developmental toxicity study as showing a LOEL/NOEL of 50/25 mg/kg/day. However, it must be recognized that the DER concluded this conditionally upon receipt of Appendix III (DER p. 7), which contains individual animal data and was not included with the study MRID. This Appendix was submitted later as part of MRID 40812001, which includes the full study as well. I am not certain whether this individual data was evaluated by anyone in HED. It was explained in the Der (p. 6) that the non-statistically significant maternal body weight gain decrease at the low dose (25 mg/kg/day) could not be adequately evaluated due to the absence of individual animal data located in the missing Appendix III. As cited in the HIDR (p. 3), mean body weight gain during days 6-18 of gestation were 0.19, 0.06, - 0.03 and - 0.03 kg at 0, 25, 50 and 100 mg/kg/day, respectively. In order to evaluate statistically the numerical decrease at the low dose level vs. Control, i.e. 0.06 vs 0.19 kg, the individual data would be needed. Furthermore, the DER claims that the decrease seen at the low dose was principally accounted for during days 6-12 and that during days 12-18 the low dose dams actually gained more weight than controls. According to the study report, body weight gain during gestation days 6-12 were 0.08, -0.04, -0.02 and -0.06 kg for control, 25, 50 and 100 mg/kg groups, respectively, where none of the dosed groups were reported as statistically significant with respect to control. (MRID table 3, p. 18).

In my opinion the data should be more closely examined before concluding where the LOEL/NOEL lies in this study, particularly if this end point is to serve as the basis for acute dietary risk assessment.

The HIDR says that there were no decreases in body weight gain at 50 mg/kg/day in the Range-Finding study. (P. 5). However, inspection of doe body weight gain data in the range-finding study shows body weight was not significantly altered at any dose level up to and including the highest dose of 400 mg/kg. (MRID 152569, table 3, p.16). Evidently, the reasons for this lack of a finding of an effect on body weight gain include the small number of animals employed and the high variability in body weight data. I do not see how this data can be cited in support of any conclusion with respect to effects of the test material on doe body weight. Furthermore, before concluding that a single dose as high as 50 mg/kg would not elicit a meaningful biological effect one should have cholinesterase data over several days following that single dose. In a journal publication mentioned in DER #11, p. 11 provided the Committee, it is noteworthy that as assessed in the Sprague-Dawley rat where malathion (American Cyanamid 95% t.a.i) were administered intraperitoneally at single doses of 0, 25, 50, 100 or 150 mg/kg, avoidance behavior was significantly impaired 1 hour after injection with 50 mg/kg and above. There were no clinical signs observed over a 24-hour post-dosing period

at any dose excepting one rat in ten at the 150 mg/kg group, which exhibited tremors. Cholinesterase inhibition was significantly inhibited only at 100 and 150 mg/kg during the 24-hour period, so the author concluded that low doses of malathion may disrupt behavior without significantly reducing cholinesterase activity [Kurtz, P. J. (1977) Dissociated Behavioral and Cholinesterase Decrements following Malathion Exposure, Toxicol. Appl. Pharmacol. 42, 589-594]. The behavioral effect found in this study was remarkable as observed at the 1 hour post-dosing time point, but was not observed at 4 or 24 hour time points.

I do not accept that a developmental toxicity study provides sufficiently rigorous toxicologic data to serve as the basis for defining this critical end point. The absence of cholinesterase assessments in particular in these studies should preclude their use as the primary source of information for an end point as important as that for use in acute dietary risk assessment.

Acute Dietary Risk Assessment

The HIDR claims that the 10X factor to account for increased sensitivity of infants and children required under FQPA should be removed. This is rationalized on the grounds there is no evidence in the reproduction and developmental toxicity studies of increased sensitivity of developing and young animals. In the rabbit developmental toxicity study doses administered during gestational days 6-18 were 0, 25, 50 and 100 mg/kg/day. Similarly in the rat developmental toxicity study (MRID 41160901) doses administered during gestational days 6-15 were 0, 200, 400 and 800 mg/kg/day. We concur that in neither of these studies was there any evidence of increased sensitivity of the developing organisms with respect to the dams, insofar as the parameters evaluated were concerned. There is a serious question, however, whether such parameters are adequate to detect critical end points. The lowest dose used in both of these studies are well above those that inhibit cholinesterase in adult rats and rabbits. In the absence of cholinesterase assessments or clinical signs in the developing organisms versus those of the maternal animals, it is simply not possible to affirm that the developing organisms were not more adversely affected than the maternal animal. I am of the opinion that cholinesterase inhibition could have been more remarkably inhibited in selected developing tissue of fetuses, and furthermore, a given level of inhibition may be more deleterious in various ways in developing organisms that would not be found in the limited set of end points evaluated in developmental toxicity studies. On the face of it, though the developmental toxicity study is useful in detecting possible developmental anomalies, its capability is not sufficient to address possible cholinergic effects or cholinesterase inhibition, as these very fundamentally important parameters are simply not evaluated.

In the case of the reproduction study (MRID 41583401) concentrations administered via the diet for two generations were 0, 550, 1700,5000 and 75000 ppm. The low dose concentration in this study translates to 43 mg/kg/day for males and 51 mg/kg/day for females. The HIDR states that pups were no more sensitive than adults on the basis of such parameters as body weight, mortally, clinical signs. It is my observation that doses of 43-51 mg/kg/day and above would have resulted in cholinesterase inhibition, given the facts that the enzyme has been shown in other subchronic studies or time intervals to be inhibited at much lower doses, in fact. It is not particularly surprising that clinical signs were not observed except at the highest dose. In terms of clinical signs, rats tolerate cholineserase inhibition borne of malathion exposure remarkably well. As in the case of the developmental toxicity studies, the question is whether a differential inhibition between pups/young animals and adults would have been observed, and whether young individuals are more or less sensitive in terms of behavioral effects (a term that embraces many types of end points). These parameters are not evaluated in these types of studies. So I must reiterate the opinion that developmental and reproduction studies while perhaps adequate to assess the effects of chemicals on the parameters of primary interest in those studies, namely developmental and reproductive effects, such studies are not of the character needed to differentiate relative sensitivity of young and mature animals to satisfy FQPA concerns. The absence of cholinesterase assessments is a most fundamental road block for this use of these studies. The elimination of

the 10X factor cannot be justified except on crude and therefore risky terms from the public health perspective. There is evidence from various studies that young and developing animals have an enhanced sensitivity to cholinesterase inhibitors in general, attributable to cholinesterase inhibition [Pope, C. N. and Chakraborti, T. K. (1992) Dose-Related inhibition of brain and plasma cholinsterase in neonatal and adult rats following sublethal organophosphate exposures. Toxicol. 73, 35-43]. Therefore, there is incumbency to demonstrate that young animals are not more sensitive than adults to the effects of malathion on that very basis, namely, cholinesterase inhibition and behavioral consequences, which were not assayed in the very studies cited to rule out the possibility of greater sensitivity of young individuals.

It is a curiosity that in HIDR pp. 13-14 under the topic of Determination of Sensitivity, mention is made of the fact that cholinesterase data were not obtained for maternal animals nor their offspring or fetuses in the reproduction and developmental toxicity studies, without any attendant discussion of the implications of this lack of data. I believe the implications are precisely those expressed above, which is that without such data it cannot be said that young animals are no less sensitive than adults to the effects of malathion, and, hence, the elimination of the FQPA required 10X factor would be without justification.

B. Chronic Dietary [Reference Dose (RfD)]: This portion of HIDR shows the calculation of an RfD based upon plasma cholinesterase inhibition in the recent F344 rat chronic toxicity/carcinogenicity study (MRID 43942901). The problem I have with this is that it does not address the failure of that study to identify a NOEL for erythrocyte cholinesterase inhibition among females during the first three months of testing. My arguments are discussed in my November 10, 1997 memorandum to Clark Swentzel, Chairman of this Committee. I will not take the time to reiterate those views here, except to emphasize the importance of obtaining a definitive NOEL for cholinesterase inhibition as explained in the memo cited. Given the facts that erythrocyte cholinesterase was inhibited in female rats at 100 ppm and 500 ppm at the three month time point, but not at the 50 ppm or 500 ppm levels at the six month time point is inexplicable. Possible explanations are that there is adaptive recovery post three months (in which case 50 ppm is not a definitive NOEL for that initial three month period, a critical time frame) and too few animals were employed to obtain good cholinesterase data in view of the shallow dose response for malathion. Such possible explanations support conducting a definitive cholinesterase assessment over a three month time point using adequate numbers of rats to provide statistical resolution. Another <u>possible</u> explanation is flawed cholinesterase methodology, which if true may be a more fundamental problem not peculiar to malathion. The point is that until a NOEL for cholinesterase inhibition among females has been determined via a definitive study, the transfer of the RfD from the Moeller and Rider study in my opinion lacks adequate support.

The HIDR (p. 6) claims that the NOEL of the 2-year study is supported by the 90-day study. If this is in reference to the subchronic neurotoxicity study (MRID 43269501), it is true a NOEL of 50 ppm was found over the 90-day period, but that study employed but -5 rats/sex/group at each time point and had no other dose group between 50ppm and 5000 ppm that would demonstrate the ability of the study to detect cholinesterase inhibition within that large range. Furthermore, plasma cholinesterase inhibition is so imprecise in that study that it is questionable whether 5000 ppm or even 50 ppm is a NOEL in either sex, which underscore the need for a study on a large number of animals to obtain a definitive NOEL for cholinesterase inhibition.

In the mouse carcinogenicity study (MRID 43407201) there is no <u>NOEL</u> for liver histopathology in male mice, where the LOEL is 100 ppm (17.4 mg/kg/day). This study awaits a Pathology Work Group evaluation.

<u>Chronic Dietary Risk Assessment</u> : HIDR (p. 6) says that the Committee determined that the 10X factor should be removed. The reasons cited are the same as those for dropping the 10X factor from the acute risk

assessment, namely the reproduction and developmental toxicity studies do not show a greater sensitivity of offspring or fetuses. To this I respond with the same arguments presented above in the case of the acute risk assessment, which is that it is not justified.

C. Occupational/Residential Exposure

1. **Dermal Absorption:** O.K.

2. **Short-Term Dermal - (1-7 days)** : O.K.

3. **Intermediate-Term Dermal (7 Days to Several Months)** : O.K.

4. **Long-Term Dermal (Seven Months to Life-Time) :** O.K.

5. **Inhalation Exposure (Any-Time Period):** The executive summary provided for the subchronic inhalation study is correct. I should emphasize that hyperplasia of the olfactory epithelium was described as locally extensive and that the olfactory/respiratory epithelial junction was severely affected in most animals. This means at all doses and there was no NOEL. The HIDR claims that since this study is the only inhalation study available in the toxicology data base, the LOEL will be used for short - intermediate - and chronic inhalation risk assessment. I view this as quite a burden for a study without a NOEL for both cholinesterase inhibition and nasal hyperplasia, but I have the greater concern for the hyperplasia aspect. **It is my opinion that this Committee should mandate a new inhalation study designed to identify a NOEL for histopathology of nasal tissues.** I say this not only because there was no NOEL, but because the hyperplasia is described as severe. There is a rational basis for a remarkable effect of malathion in particular on the olfactory epithelium, which is discussed at length in the DER for the recent malathion F344 chronic toxicity/carcinogenicity study (MRID 43942901). Briefly, the sensitivity of the olfactory epithelium to malathion rests with the remarkable metabolic capability of this tissue, as well as the unique structure of malathion as a diester of a dicarboxylic acid which may be hydrolyzed in the olfactory epithelium to yield carboxylic acids. The metabolic capability of the olfactory epithelium has been hypothesized as critical to the maintenance of acuteness of olfaction via the elimination of foreign materials including odorants. Given these factors which may explain the remarkable effect of malathion on the olfactory epithelium, in concert with the severity of the effect, as well as not knowing the time of onset of hyperplasia, I consider the application of a mere UF of 3 to cover for the lack of a NOEL to be entirely inadequate. I say this in view of both the smallness of the UF chosen, and an operating philosophy which in lieu of weighing the significance of the finding, simply invokes a UF without offering any explanation as to why 3 is adequate, or why another study should not be required.

The April 27, 1995 HED memorandum conveying the DER to the Product Manager says among other things: "The question of carcinogenicity as it may relate to the microscopic lesions of the nose and larynx will be addressed in a separate memorandum." To my knowledge such a memorandum remains outstanding, and this very important issue has not been addressed.

D **Margin of Exposure for Occupational/Residential Exposures**

(1) MOE for Dermal Exposures: see comments as before on the use of reproduction and developmental toxicity studies to rule out the possibility of enhanced sensitivity of young animals.

(2) MOE for Inhalation Exposures: As stated above, I do not support the use of the UF of 3. Again I find unmerited the claim that:"No FQPA factors are required since there was no indication of increased sensitivity

in the offspring of rats or rabbits to prenatal exposure to malathion.", lacking cholinesterase data or behavioral effects assessments.

E **Recommendation for Aggregate Exposure Risk Assessments**
No additional comments

III. FQPA Considerations

1. **Neurotoxicity Data**
In the case of the acute neurotoxicity study, concerning bilateral retinal rosette observed in one male rat, the statement might be improved somewhat in its meaning by saying that the one rat in which it was observed was from among but five males examined histopathologically in the high dose group, and that none were examined in lower dose groups. Also, concerning the acute and subchronic neurotoxicity studies mentioned, I would cite my memorandum of November 20, 1997 to Clark Swentzel as detailing comments I might otherwise offer here.

2. Determination of Sensitivity
No further comments on the developmental and reproduction studies.

VII **Data Gap(s)**

Roman numerals go from III to VII in the HIDR.

From my perspective, the following are data gaps:

1. Carcinogenicity Study in B6C3F1 Mice (MRID 43407201) :
Pathology Working Group assessment for liver tumors;
Histopathology assessment of nasal tissues.

2. Combined Chronic Toxicity/Carcinogenicity F344 Rat Study (MRID 43942901) : Pathology evaluation/reevaluations of various tissues.

3. Subchronic Inhalation Study in Sprague-Dawley Rat (MRID 43266601) : resolution of no NOEL for nasal tissue histopathology, which was severe at the lowest dose and present in essentially all rats of both sexes; recommend a new and longer term study to address the absence of a NOEL and potential carcinogenicity by the inhalational route.

4. Developmental Toxicity Study in the Rabbit (MRID 152569) : submission of Appendix III followed by statistical treatment of the individual data to affirm the NOEL for body weight effects in dams particularly over days 6-12 of gestation.

5. Acute Neurotoxicity Study in the F344 Rat (MRID 43146701): submission of selected retinal tissue slides as called for in the DER.

6. Subchronic Neurotoxicity Study in the F344 Rat (MRID 43269501) : submission of a guideline behavioral test yet to be specified.

7. Three-month cholinesterase assay in the rat to determine a definitive LOEL/NOEL for malathion.

Brian Dementi, Ph.D

Toxicologist/HED

ATTACHMENT 3: Letter from B. Dementi - November 10, 1997

Clark Swentzel, Chairman
HazardID SARC
Health Effects Division

November 10, 1997

As a follow-up to the November 6, 1997 HazardID SARC on malathion, I am compelled to express in writing my disagreement with certain very important decisions rendered at that meeting. One such issue concerns the apparent decision of the Committee to shift the basis of the RfD for malathion from the NOEL in the human study (Moeller and Rider, 1962), which has served in this capacity for years, to the NOEL for cholinesterase inhibition in the 1996 F344 rat chronic toxicity/carcinogenicity study. The problems I have with this decision are developed as follows. Firstly, the decision was too precipitous. By this I mean that since this is such a critical end point for this pesticide, it should have been presented as an issue or topic well before the meeting to allow people to be better prepared for discussion. I view this as a problem inherent in the process in dealing with a chemical having an extensive scientific record. Accordingly, there must be opportunity for offering further arguments supportable by additional information.

To the extent that Moeller and Rider incorporates a valid assessment of the LOEL/NOEL for cholinesterase inhibition in human subjects, being based as it is on **both** plasma and erythrocyte cholinesterases, evidence suggests humans are at least 10-fold more sensitive than F344 rats for erythrocyte cholinesterase inhibition and even more sensitive with respect to the plasma enzyme. To explain this difference, someone at the meeting suggested that 1962 vintage malathion was of questionable purity and that impurities could explain the differences with respect to the 1996 product. However, it was not indicated that humans have historically been more sensitive, i.e. were more sensitive than rat as compared on the basis of earlier products and likely remain so as compared to the more recent Cheminova product. Critical to the sensitivity of organisms to malathion in the cholinergic sense is the presence and level in such organisms of carboxylesterase activity, an enzyme(s) which, via catalysis of hydrolysis of one carboxyethyl group on malathion (actually malaoxon as the cholinesterase inhibiting entity), compromises its cholinesterase inhibitory capabilities. As I indicated at the meeting, insects lack carboxylesterase activity, which is thought to explain the remarkable selective efficacy of malathion as an insecticide. Similarly, to the extent that mammals incorporate differential levels of carboxylesterase activity they are variably sensitive to the agent in the cholinergic sense. Published works show that while carboxylesterase activity is located in the plasma and liver of the rat, in humans the enzyme is found in liver but not plasma. (Exhibit 1) The greater sensitivity of humans as demonstrated in Moeller and Rider may have its explanation in differing carboxylesterase activity in man versus rat. However, whatever the explanation, the fact remains that Moeller and Rider demonstrates the greater sensitivity of humans as compared historically using malathion of existing purity at the time and would likely prove so today if compared using the recent Cheminova product. I present these views as a way of dismissing any notions that Moeller and Rider has any fundamental flaw, if it can be accepted that malathion used in that study was at least as pure as 1962 vintage technical malathion, though purity of malathion used in the study was not provided. If it were a more highly purified product, then to the extent that such culprit cholinesterase inhibiting impurities as malaoxon and isomalathion were reduced, the concern about relative human sensitivity would be to that extent more enhanced.

In view of these considerations, greater scrutiny of the rat cholinesterase data than was had at the November 6 meeting would be essential before a shift could be made from human to rat data as the basis for deriving an RfD. Along these lines I have the following to say. The Cheminova malathion technical product is said to be more pure than the former American Cyanamid product. Before the Committee accepts such claim, members should have in hand the Confidential Statement of Formulation for the respective products for direct comparison by the Committee. This is particularly important with respect to levels of cholinesterase inhibiting impurities. Cheminova has submitted data showing higher LD50 values for their product versus the American Cyanamide product, but LD50 may not be a good reflection of how products may compare at low levels of exposure based on cholinesterase data. LD50 values may be confounded by a host of adverse effects of the test material including cholinesterase inhibition brought on by trace impurities of cholinesterase inhibiting entities that do not require activation and thus become relatively more important at high doses of malathion where metabolic conversion of malathion to malaoxon becomes more saturated. Actually, I must confess to the committee that I very carefully compared the two product compositions awhile ago and there are reduced levels of malaoxon and isomalathion in the Cheminova product versus the American Cyanamid product, but I would question the relative effects of these these entities at low doses where metabolic conversion of malathion to malaoxon is less saturated.

In developing the protocol for the recently (1996) submitted malathion chronic/carcinogenicity study, the registrant was advised by our staff that 100 ppm, which the registrant was proposing as a low dose for the study, included principally in search of a NOEL for cholinesterase inhibition, would likely not be a NOEL for the blood borne cholinesterases. (Exhibit 2) It was explained that 100 ppm (lowest dose tested) was not a NOEL in the 1980 chronic/carcinogenicity study in the Sprague-Dawley rat, and likely would not be a NOEL in the new study. Nontheless, the registrant elected 100 ppm as the low dose for the new study, partly predicated on their view that their product is more pure than the American Cyanamide product empolyed in the earlier studies. As it developed, after 3 months on test, statistically significant erythrocyte cholinesterase inhibition was observed in females, prompting a reduction of the low dose to 50 ppm for rats of both sexes for the duration of the two year study in search of a NOEL. (Exhibit 3) I should note at this point that this finding corroborated the finding in the Sprague-Dawley rat performed seventeen years ago using tha American Cyanamid product. Subsequent to the three month time point, 50 ppm proved to be a NOEL for erythrocyte cholinesterase for both sexes. Firstly, what this says to me is that there is little if any improvement in the Cheminova product over that of the American Cyanamide product with respect to inhibition of erythrocyte cholinestyerase at low doses, particularly those critical to setting the RfD for malathion. Secondly, in the DER for the new chronic/carcinogenicity study in the rat, additional cholinesterase information is called for in view of the absence of a NOEL for cholinesterase inhibition among females at the 3 month time point. It is alleged in the DER that given the ability of organisms to adapt somewhat to cholinesterase inhibitors (see, for example, the recovery of erythrocyte cholinesterase inhibition for females at 500 ppm at 6 months in that study, Exhibit 4), there is no assurance that the enzyme would not have been inhibited at 50 ppm during the first three months, i.e. during a very critical time frame for exposure to a pesticide. This is also very important in view of the facts that, a) malathion has a very shallow dose response curve (in my judgement there is very little difference between 50 and 100 ppm for an agent that demonstrates such a shallow dose response curve ranging up to 6000-12000 ppm), b) the human study demonstrated greater sensitivity for uncertain reasons and c) the number of animals assayed for cholinesterase activity, 10/sex, does not accord sufficient statistical power to clearly identify a NOEL at low but meaningful levels of inhibition. I must maintain at this point that a definitive NOEL for cholinesterase inhibition be determined over at least a three month period using large numbers of rats at doses that embrace those employed in Moeller and Rider (.11-.34 mg/kg/day) overlapping those of the lower dose range of the rat chronic/carcinogenicity study, say up to 20 mg/kg/day. To the extent that this end point will be employed in establishing the RfD for malathion, I view it imperative that this data be gathered.

In summary I consider it inappropriate to change the basis of the RfD for malathion from the Moeller and Rider human study to the recently submitted chronic toxicity/carcinogenicity study in the F344 rat, particularly without a definitive NOEL for cholinesterase inhibition over the first three months of testing in ths case of the rat. Also, I recommend additional study to obtain a more definitive NOEL for cholinestarase inhibition at low doses in the rat

cc Jess Rowland

Brian Dementi
Toxicologist, HED.

ATTACHMENT 4: Letter from B. Dementi - November 20, 1997

Clark Swentzel, Chairman
Hazard ID SARC
Health Effects Division

November 20, 1997

Re: Ad Hoc Committee Meeting of November 13, 1997 on Malathion Issues

As a matter of the record, regarding the referenced meeting, this is to advise you that in spite of the good effort on your part to see that a fair and reasonable meeting was held, and I thought you did well, I do not consider the outcome satisfactory. The decisions made were very inadequate and not in the interest of the public health, as they compromise full pursuit of the understanding of the toxicology profile on this important and extensively used pesticide. No stone should be left unturned, given the enormity of human exposure to this cholinesterase inhibiting organophosphate. I shall comment on the topics that were the subject of the meeting in the order in which they were taken.

Retinal Anomaly in Acute Neurotoxicity Study on Malathion (MRID 43146701

I have presented fully my views on this subject in written documents, which were available to the Committee, and will not restate these views at this writing. The fact remains that the Acute Neurotoxicity Guidelines (81-8) call for sequential histopathologic evaluations of specific tissues in lower dose groups when histopathologic findings are noted in the high dose group animals. It would appear to me that this requirement should be met in this Guideline even if but one lesion is observed in a particular tissue of the high dose group given the small number of animals (5/sex) in a dose group. This was not done in the study in question after the one bilateral retinal rosette was noted in a high dose male group. Now it is not a source of happiness to me to be perceived as one who over-assesses a study, and this is why I feel very awkward in defending this position. If the one incident standing alone had been identified among fifty or more animals in a group, surely I would not have pursued the matter, but in this case given the rarity of the lesion in historical data bases and the uncertainty as to the lesions microscopic anatomic features (retinal rosette is not an anatomic term and on the face of it, the term could be used to apply to any of a variety of underlying morphologic changes), I felt that as a matter of the record, our pathologist should provide anatomic characterization. Also, there was somewhat greater incumbency to require this assessment since it involved the retina, in view of the prevailing concerns over possible retinal effects of organophosphates in general and of malathion in particular. While I did not say so at the November 13 meeting, it is essentially self-evident that the assesment of the requested slides could be instrumental in determining whether to insist upon examinations of lower dose groups as mandated in the Guidelines. For example, this might be contingent upon whether the bilateral retinal rosette of the high dose male in the acute study is morphologically or anatomically the same as that of the unilateral rosette of a control rat in the subchronic neurotoxicity study.

Lastly, I believe the relatively minor decision to ask for a couple slides should be entirely within the perview of the reviewer, given what may be his peculiar perspectives on the subject, without having it go before a committee for approval. As I said, for the record, this issue remains unresolved if the slides in question are not submitted.

Relative Sensitivity of Females Versus Males to Cholinesterase Inhibition by Malathion

I presented to the Committee several comparisons of the level of cholinesterase inhibition for males and females from our Guideline and dose range-finding studies on malathion and malaoxon. Although the magnitude of differences between the sexes is variable across studies, there is more than adequate evidence to establish a greater sensitivity for females. The ad hoc Committee did agree that sex-related differences are manifest, but did not concur with the proposition that differences may merit a correction factor to be applied to male (human) data used as the basis for the RfD. It should be noted at this point that the RfD for malathion, 0.02 mg/kg/day, which ostensibly protects the entire human population - men, women, boys and girls of all ages- employs a mere ten-fold safety factor as applied to experimental data obtained on humans (men only). In the absence of such data for women and youths, in my judgement a larger safety factor than ten should be employed, particularly in the face of evidence that females are more sensitive to malathion than males as assessed in laboratory animal studies, and where studies of organophosphates in general suggest young individuals to be more sensitive. According to the 1988 malathion registration standard: "The Theoretical Maximum Residue Contribution (TMRC) for the U.S. population average is 0.1014 mg/kg/day, occupying 505% of the PADI. For children 1 to 6 years of age, the TMRC occupies 1133% of the PADI. The TMRC is based upon current tolerance levels and an assumption that 100% of the sites are treated. Actual dietary exposure may be much lower." (p.32) The point is that a much higher percentage of the PADI is consumed, or was so in 1988, than is to be desired, which places an enhanced scrutiny upon the reliability of the RfD in protecting real people.

Unfortunately I did not have the time before the meeting to provide study by study estimates of such correction factors, but am certain that a legitimate correction factor, whatever it is, would be of such magnitude that it should not be ignored, especially in view of the small safety factor used for the existing RfD. Additional study in animals may be necessary to properly identify the correction factor. Realizing that a sex-related differential sensitivity exists, unacceptable in my opinion is the Committee's out of hand rejection of the argument that a meaningful ratio exists without first obtaining some numerical estimates of that ratio of sensitivity from the data currently in hand. Indeed, I had anticipitated that an outcome of the meeting would be a Committee recommendation that such estimates be computed for subsequent consideration.

Testing for Effects on Learning/Memory

Again, available to the Committee were various documents presenting arguments pro and con that findings with malathion on learning/memory at very low doses in a published work, Desi et al. (1976), are of sufficient validity and concern to require Guideline testing of malathion for these effects. In addition to explaining to the Committee that the published work shows that malathion at doses of 38-75 mg/kg/day in a subchronic study elicited effects on learning/memory, EEG and EMG, as contrasted with no neurotoxic (motor activity, FOB parameters) effects in the Guideline subchronic neurotoxicity study at doses up to 1575 mg/kg/day, I had recommended that a Guideline test of learning/memory be required for malathion. The Committee rejected this recommendation on the grounds that Desi et al (1976) is not a reliable study. This criticism of the study was maintained in spite of many findings in the study that affirm its veracity. Of these I mentioned the facts that the stated purpose of the authors was to assess the effects of malathion at subclinical levels on sensitive neurotoxicity parameters including learning/memory; 95% malathion (American Cyanamid) was used; the authors affirmed the absence of clinical signs which was consistent with the low but meaningful level of cholinesterase inhibition; cholinesterase activity was remarkably well evaluated in the study, including assessments of plasma, erythrocytes and brain regions, where the findings were consistent with those of the Guideline subchronic neurotoxicity study (which in turn enhances the credibility of the published work), and adverse effects of malathion on kidney tissue in in vitro kidney tissue cultures being somewhat consistent with or supported by chronic nephropathy as the cause of increased mortality (100% and 74% in the high and penultimate doses, respectively) in the 1996 chronic toxicity/carcinogenicity study in the F344 rat. Furthermore, the authors of the study affirm in the text a real effect of malathion on learning and memory as

assessed in their study.

The Committee members were mute with respect to acknowledging any of these facts as supporting evidence of the work by Desi et al, but persisted in criticizing the study on the grounds that the effects on learning/memory in terms of errors made by rats in maze studies were small, not dose related between 38 and 75 mg/kg/day; that statistics were ill defined and that it would be surprising for malathion to exert such an effect at such low dose levels. I endeavored to explain that findings were in fact not small in terms of differences in errors made in dosed groups versus controls. I also offered my opinion that 38 and 75 mg/kg/day, when compared on the shallow dose response for malathion are actually not very different, and that brain cholinesterase inhibition was 20% in the two groups at 21 days, the time at which learning/memory was affected. These two observations would point to similar responses on tests of learning/memory, and thus the absence of a dose response as noted. I also explained from an earlier work by Desi et al, which the authors cited as background for methodology, that bar graphs in that study were said to be standard deviations, which if true in the 1976 study would mean that differences between controls and dosed groups on errors made in the learning /memory test would be statistically significant. In spite of these findings, plus the EEG and EMG data affirming a neurological effect of the test material at these dose levels, and in view of the fact that the Guideline subchronic neurotoxicity study was not designed to assess learning/memory, EEG or EMG effects that could refute the findings in Desi et al, the Committee categorically rejected the Desi study as of any relevance. In fact, I recall saying to the group, "It's as if Desi does not exist?", whereupon I was responded to in the affirmative. In my judgement, this qualifies as an authoritarian rejection of data the Committee failed to refute. I maintain that Desi et al (1976) in spite of its deficiencies is of sufficient quality that it conclusions, particularly with respect to the effects of malathion on learning/memory, mandate verification through proper Guideline testing procedures, which are available. As to the question of the "small" effect on errors made by rats in the learning and memory aspect of Desi, et al, one might ask, what is small? Imagine a high school student taking his algebra exam, on which his grade would be say 97, other things being equal, but under the influence of a xenobiotic he was exposed to, his score turned out to be 92 due to a few additional errors he made. Now a 92 (B) is a very good grade, but not quite as good as the grade he deserved 97 (A). One might say this is a small difference, but who would argue that is to be ignored?

I have concerns about the legitimacy of the opportunity presented to me to go before an unbiased ad hoc committee. I had reservations before the November 13 meeting that I should even pursue the matter. This concern was born out by the following episode that occurred at the meeting. As you will recall during the meeting, at the precise moment that we completed our deliberations on the second topic, one Committee member, arriving late, voted on the issue. In fact, as I recall, you commented at the time that so and so is voting even though she was not present during the discussion. From my perspective, her vote was more than improper in that it conveyed the impression, whether rightly or wrongly interpreted, that the Committee's conclusions were foreordained, and that my opportunity to be heard at this meeting was a mere formality. When I came to item three, my presentation was compromised in the psychological or motivational sense, given what had previously taken place. I could see "The handwriting on the wall" and thus the futility in proceeding further on what was really the most important of the three issues. In my view, minds had been made up, and I felt nothing I said would matter before this Committee. Indeed, I came preciously close to calling off any further discussion, but felt that would be of no avail either, as people might then say "well, you had your chance", as if this were some kind of real and legitimate peer review. I am convinced it was so in name only. The bottom line to all this is that another forum for peer review of these issues is required, bearing in mind the importance of this subject to the public health. People composing a true peer review committee should be experts in the field, but at the same time should not have personal vested interest in HED.

Brian Dementi
Toxicologist, HED
cc Jess Rowland

ATTACHMENT 5: Letter from B. Dementi - November 25, 1997

Clark Swentzel, Chairman
Hazard ID Committee

November 25, 1997

RE: Malathion RfD

It is my intent here to comment further on certain issues before the Hazard ID SARC of November 6 and the Ad Hoc Committee meeting of November 13, 1997, with particular reference to the RfD for malathion.

In my memorandum to you of November 10, I endeavored to explain why the cholinesterase data in the recent chronic toxicity/carcinogenicity study of malathion is inadequate to define a NOEL for female F344 rats. As a remedy, I recommended a definitive three month assessment of cholinesterase inhibition in the rat. In my judgement, until such data are available, a gap exists with respect to the identification of a NOEL for the first three months of exposure to malathion, and, hence, proper data do not exist in this study upon which to poise an RfD. This being true, and to the extent that the Moeller and Rider (1962) study, performed in humans, may continue to be used as the basis for the RfD until proper rat data are obtained, the following comments are relevant.

At the Ad Hoc Committee meeting, when discussing the topic of greater sensitivity of females to cholinesterase inhibition by malathion, I expressed the view that for studies wherein cholinesterase inhibition was obtained in but one sex, as is true in Moeller and Rider where only male volunteers were tested, that a greater than the normal uncertainty factor (UF) of 10 should be applied. As I recall, this was not affirmed by any one at the meeting. I suspect no one felt sufficiently certain to render a definite opinion. In any case, I believe this is a question requiring an answer. I do not have the time to search the records, but I believe the answer should be readily available in the minutes of past RfD meetings, and should be a well recognized operating principle for the RfD Committee. I have just by chance reviewed the 1997 Registration Eligebility Document (RED) toxicology chapter for carbofuran, and I find in the case of the RfD that the Agency applied a UF of 100 to the NOEL for cholinesterase inhibition in male volunteers. Quoting from that RED chapter: "An uncertainty factor (UF) of 10 was applied to account for intra-species variability. An additional UF of 10 was applied to account for study deficiencies (use of limited number of subjects, few subjects/dose and **use of males only** (emphasis added)". Please be aware that Moeller and Rider, in addition to being a study in males only, has its inadequacies also (e.g., limited number of subjects, purity of the test material not provided, interpretation of low and mid dose effects somewhat confounded by co-administration of EPN).

In my memorandum to you of November 20, I quoted from the malathion registration standard, passages revealing how high the TMRC is (or was in 1988) when based on the RfD of 0.02 mg/kg/day, derived from Moeller and Rider with a UF of only 10. The Committee should be aware that at an earlier time point, a UF of 100 had been applied to Moeller and Rider, at which time the RfD was thus 0.002 mg/kg/day. Also at that time the TMRC was about 5000% of the PADI. At some point in time, and I don't have the details, I would estimate around 1987-90, the UF was reduced from 100 to 10, for reasons unknown to me. I recommend that your Committee seek the historical record on the setting of the RfD for malathion, and make your own independent assessment of its reasonableness, as this is the moment in time for reconciling the RfD with the facts at hand. On the face of it, if a UF of 100 is appropriate for carbofuran for the reasons given, an explanation should be forth coming for the use of only 10 in the case of malathion. Please understand I am not saying a satisfactory explanation does not exist, but let us see it. I must maintain the view that when a UF of only 10 is employed, it is imperative that the study in question incorporate data on both sexes.

In summary, in my view proper data on cholinesterase inhibition in rats are not available at this moment to justify replacing the Moeller and Rider human study as the basis for the RfD for malathion. Furthermore, in the absence of cholinesterase data on women, the UF as applied to the Moeller and Rider human (men only) data should be revised upward from the 10 which is currently employed.

Brian Dementi, Ph.D.
Toxicologist/HED

cc Jess Rowland
George Ghali

ATTACHMENT 6: Letter from B. Dementi - December 17, 1997

Jess Rowland, Secretary
Hazard ID Committee

December 17, 1997

Comments on December 4, 1997 draft report of malathion Hazard ID Committee meeting of November 6, 1997. The following is the best I am able to produce given the constraints of time and the complexity of the subject.

Comments on the various endpoints are presented as follows in the order in which they appear in the draft report.

I Introduction (p. 1) O.K.

11 Hazard Identification

A. Acute Oral (one-day): For this endpoint, the Committee concluded that the 50 mg/kg/day dose is appropriate for acute dietary risk assessment. This endpoint is based upon decreased maternal body weight gain in the malathion developmental toxicity study in the rabbit (MRID 152569). In support of this, the draft Hazid ID Committee Report (HIDR) cites the DER for the rabbit developmental toxicity study as showing a LOEL/NOEL of 50/25 mg/kg/day. However, it must be recognized that the DER concluded this conditionally upon receipt of Appendix III (DER p. 7), which contains individual animal data and was not included with the study MRID. This Appendix was submitted later as part of MRID 40812001, which includes the full study as well. I am not certain whether this individual data was evaluated by anyone in HED. It was explained in the Der (p. 6) that the non-statistically significant maternal body weight gain decrease at the low dose (25 mg/kg/day) could not be adequately evaluated due to the absence of individual animal data located in the missing Appendix III. As cited in the HIDR (p. 3), mean body weight gain during days 6-18 of gestation were 0.19, 0.06, - 0.03 and - 0.03 kg at 0, 25, 50 and 100 mg/kg/day, respectively. In order to evaluate statistically the numerical decrease at the low dose level vs. Control, i.e. 0.06 vs 0.19 kg, the individual data would be needed. Furthermore, the DER claims that the decrease seen at the low dose was principally accounted for during days 6-12 and that during days 12-18 the low dose dams actually gained more weight than controls. According to the study report, body weight gain during gestation days 6-12 were 0.08, -0.04, -0.02 and -0.06 kg for control, 25, 50 and 100 mg/kg groups, respectively, where none of the dosed groups were reported as statistically significant with respect to control. (MRID table 3, p. 18).

In my opinion the data should be more closely examined before concluding where the LOEL/NOEL lies in this study, particularly if this end point is to serve as the basis for acute dietary risk assessment.

The HIDR says that there were no decreases in body weight gain at 50 mg/kg/day in the Range-Finding study. (P. 5). However, inspection of doe body weight gain data in the range-finding study shows body weight was not significantly altered at any dose level up to and including the highest dose of 400 mg/kg. (MRID 152569, table 3, p.16). Evidently, the reasons for this lack of a finding of an effect on body weight gain include the small number of animals employed and the high variability in body weight data. I do not see how this data can be cited in support of any conclusion with respect to effects of the test material on doe body weight. Furthermore, before concluding that a single dose as high as 50 mg/kg would not elicit a meaningful biological effect one should have cholinesterase data over several days following that single dose. In a journal publication mentioned in DER #11, p. 11 provided the Committee, it is noteworthy that as assessed in the Sprague-Dawley rat where malathion (American Cyanamid 95% t.a.i) were administered intraperitoneally at single doses of 0, 25, 50, 100 or 150 mg/kg, avoidance behavior was significantly impaired 1 hour after

injection with 50 mg/kg and above. There were no clinical signs observed over a 24-hour post-dosing period at any dose excepting one rat in ten at the 150 mg/kg group, which exhibited tremors. Cholinesterase inhibition was significantly inhibited only at 100 and 150 mg/kg during the 24-hour period, so the author concluded that low doses of malathion may disrupt behavior without significantly reducing cholinesterase activity [Kurtz, P. J. (1977) Dissociated Behavioral and Cholinesterase Decrements following Malathion Exposure, Toxicol. Appl. Pharmacol. 42, 589-594]. The behavioral effect found in this study was remarkable as observed at the 1 hour post-dosing time point, but was not observed at 4 or 24 hour time points.

I do not accept that a developmental toxicity study provides sufficiently rigorous toxicologic data to serve as the basis for defining this critical end point. The absence of cholinesterase assessments in particular in these studies should preclude their use as the primary source of information for an end point as important as that for use in acute dietary risk assessment.

Acute Dietary Risk Assessment

The HIDR claims that the 10X factor to account for increased sensitivity of infants and children required under FQPA should be removed. This is rationalized on the grounds there is no evidence in the reproduction and developmental toxicity studies of increased sensitivity of developing and young animals. In the rabbit developmental toxicity study doses administered during gestational days 6-18 were 0, 25, 50 and 100 mg/kg/day. Similarly in the rat developmental toxicity study (MRID 41160901) doses administered during gestational days 6-15 were 0, 200, 400 and 800 mg/kg/day. We concur that in neither of these studies was there any evidence of increased sensitivity of the developing organisms with respect to the dams, insofar as the parameters evaluated were concerned. There is a serious question, however, whether such parameters are adequate to detect critical end points. The lowest dose used in both of these studies are well above those that inhibit cholinesterase in adult rats and rabbits. In the absence of cholinesterase assessments or clinical signs in the developing organisms versus those of the maternal animals, it is simply not possible to affirm that the developing organisms were not more adversely affected than the maternal animal. I am of the opinion that cholinesterase inhibition could have been more remarkably inhibited in selected developing tissue of fetuses, and furthermore, a given level of inhibition may be more deleterious in various ways in developing organisms that would not be found in the limited set of end points evaluated in developmental toxicity studies. On the face of it, though the developmental toxicity study is useful in detecting possible developmental anomalies, its capability is not sufficient to address possible cholinergic effects or cholinesterase inhibition, as these very fundamentally important parameters are simply not evaluated.

In the case of the reproduction study (MRID 41583401) concentrations administered via the diet for two generations were 0, 550, 1700,5000 and 75000 ppm. The low dose concentration in this study translates to 43 mg/kg/day for males and 51 mg/kg/day for females. The HIDR states that pups were no more sensitive than adults on the basis of such parameters as body weight, mortally, clinical signs. It is my observation that doses of 43-51 mg/kg/day and above would have resulted in cholinesterase inhibition, given the facts that the enzyme has been shown in other subchronic studies or time intervals to be inhibited at much lower doses, in fact. It is not particularly surprising that clinical signs were not observed except at the highest dose. In terms of clinical signs, rats tolerate cholineserase inhibition borne of malathion exposure remarkably well. As in the case of the developmental toxicity studies, the question is whether a differential inhibition between pups/young animals and adults would have been observed, and whether young individuals are more or less sensitive in terms of behavioral effects (a term that embraces many types of end points). These parameters are not evaluated in these types of studies. So I must reiterate the opinion that developmental and reproduction studies while perhaps adequate to assess the effects of chemicals on the parameters of primary interest in those studies, namely developmental and reproductive effects, such studies are not of the character needed to differentiate relative sensitivity of young and mature animals to satisfy FQPA concerns. The absence of

cholinesterase assessments is a most fundamental road block for this use of these studies. The elimination of the 10X factor cannot be justified except on crude and therefore risky terms from the public health perspective. There is evidence from various studies that young and developing animals have an enhanced sensitivity to cholinesterase inhibitors in general, attributable to cholinesterase inhibition [Pope, C. N. and Chakraborti, T. K. (1992) Dose-Related inhibition of brain and plasma cholinsterase in neonatal and adult rats following sublethal organophosphate exposures. Toxicol. 73, 35-43]. Therefore, there is incumbency to demonstrate that young animals are not more sensitive than adults to the effects of malathion on that very basis, namely, cholinesterase inhibition and behavioral consequences, which were not assayed in the very studies cited to rule out the possibility of greater sensitivity of young individuals.

It is a curiosity that in HIDR pp. 13-14 under the topic of Determination of Sensitivity, mention is made of the fact that cholinesterase data were not obtained for maternal animals nor their offspring or fetuses in the reproduction and developmental toxicity studies, without any attendant discussion of the implications of this lack of data. I believe the implications are precisely those expressed above, which is that without such data it cannot be said that young animals are no less sensitive than adults to the effects of malathion, and, hence, the elimination of the FQPA required 10X factor would be without justification.

B. Chronic Dietary [Reference Dose (RfD)]: This portion of HIDR shows the calculation of an RfD based upon plasma cholinesterase inhibition in the recent F344 rat chronic toxicity/carcinogenicity study (MRID 43942901). The problem I have with this is that it does not address the failure of that study to identify a NOEL for erythrocyte cholinesterase inhibition among females during the first three months of testing. My arguments are discussed in my November 10, 1997 memorandum to Clark Swentzel, Chairman of this Committee. I will not take the time to reiterate those views here, except to emphasize the importance of obtaining a definitive NOEL for cholinesterase inhibition as explained in the memo cited. Given the facts that erythrocyte cholinesterase was inhibited in female rats at 100 ppm and 500 ppm at the three month time point, but not at the 50 ppm or 500 ppm levels at the six month time point is inexplicable. Possible explanations are that there is adaptive recovery post three months (in which case 50 ppm is not a definitive NOEL for that initial three month period, a critical time frame) and too few animals were employed to obtain good cholinesterase data in view of the shallow dose response for malathion. Such possible explanations support conducting a definitive cholinesterase assessment over a three month time point using adequate numbers of rats to provide statistical resolution. Another <u>possible</u> explanation is flawed cholinesterase methodology, which if true may be a more fundamental problem not peculiar to malathion. The point is that until a NOEL for cholinesterase inhibition among females has been determined via a definitive study, the transfer of the RfD from the Moeller and Rider study in my opinion lacks adequate support.

The HIDR (p. 6) claims that the NOEL of the 2-year study is supported by the 90-day study. If this is in reference to the subchronic neurotoxicity study (MRID 43269501), it is true a NOEL of 50 ppm was found over the 90-day period, but that study employed but -5 rats/sex/group at each time point and had no other dose group between 50ppm and 5000 ppm that would demonstrate the ability of the study to detect cholinesterase inhibition within that large range. Furthermore, plasma cholinesterase inhibition is so imprecise in that study that it is questionable whether 5000 ppm or even 50 ppm is a NOEL in either sex, which underscore the need for a study on a large number of animals to obtain a definitive NOEL for cholinesterase inhibition.

In the mouse carcinogenicity study (MRID 43407201) there is no <u>NOEL</u> for liver histopathology in male mice, where the LOEL is 100 ppm (17.4 mg/kg/day). This study awaits a Pathology Work Group evaluation.

<u>Chronic Dietary Risk Assessment</u> : HIDR (p. 6) says that the Committee determined that the 10X factor should be removed. The reasons cited are the same as those for dropping the 10X factor from the acute risk

assessment, namely the reproduction and developmental toxicity studies do not show a greater sensitivity of offspring or fetuses. To this I respond with the same arguments presented above in the case of the acute risk assessment, which is that it is not justified.

C. Occupational/Residential Exposure

1. **Dermal Absorption:** O.K.

2. **Short-Term Dermal - (1-7 days)** : O.K.

3. **Intermediate-Term Dermal (7 Days to Several Months)** : O.K.

4. **Long-Term Dermal (Seven Months to Life-Time) :** O.K.

5. **Inhalation Exposure (Any-Time Period):** The executive summary provided for the subchronic inhalation study is correct. I should emphasize that hyperplasia of the olfactory epithelium was described as locally extensive and that the olfactory/respiratory epithelial junction was severely affected in most animals. This means at all doses and there was no NOEL. The HIDR claims that since this study is the only inhalation study available in the toxicology data base, the LOEL will be used for short - intermediate - and chronic inhalation risk assessment. I view this as quite a burden for a study without a NOEL for both cholinesterase inhibition and nasal hyperplasia, but I have the greater concern for the hyperplasia aspect. **It is my opinion that this Committee should mandate a new inhalation study designed to identify a NOEL for histopathology of nasal tissues.** I say this not only because there was no NOEL, but because the hyperplasia is described as severe. There is a rational basis for a remarkable effect of malathion in particular on the olfactory epithelium, which is discussed at length in the DER for the recent malathion F344 chronic toxicity/carcinogenicity study (MRID 43942901). Briefly, the sensitivity of the olfactory epithelium to malathion rests with the remarkable metabolic capability of this tissue, as well as the unique structure of malathion as a diester of a dicarboxylic acid which may be hydrolyzed in the olfactory epithelium to yield carboxylic acids. The metabolic capability of the olfactory epithelium has been hypothesized as critical to the maintenance of acuteness of olfaction via the elimination of foreign materials including odorants. Given these factors which may explain the remarkable effect of malathion on the olfactory epithelium, in concert with the severity of the effect, as well as not knowing the time of onset of hyperplasia, I consider the application of a mere UF of 3 to cover for the lack of a NOEL to be entirely inadequate. I say this in view of both the smallness of the UF chosen, and an operating philosophy which in lieu of weighing the significance of the finding, simply invokes a UF without offering any explanation as to why 3 is adequate, or why another study should not be required. The April 27, 1995 HED memorandum conveying the DER to the Product Manager says among other things: "The question of carcinogenicity as it may relate to the microscopic lesions of the nose and larynx will be addressed in a separate memorandum." To my knowledge such a memorandum remains outstanding, and this very important issue has not been addressed.

D **Margin of Exposure for Occupational/Residential Exposures**

(1) MOE for Dermal Exposures: see comments as before on the use of reproduction and developmental toxicity studies to rule out the possibility of enhanced sensitivity of young animals.

(2) MOE for Inhalation Exposures: As stated above, I do not support the use of the UF of 3. Again I find unmerited the claim that:"No FQPA factors are required since there was no indication of increased sensitivity in the offspring of rats or rabbits to prenatal exposure to malathion.", lacking cholinesterase data or behavioral effects assessments.

E **Recommendation for Aggregate Exposure Risk Assessments**
No additional comments

III. FQPA Considerations

1. **Neurotoxicity Data**
In the case of the acute neurotoxicity study, concerning bilateral retinal rosette observed in one male rat, the statement might be improved somewhat in its meaning by saying that the one rat in which it was observed was from among but five males examined histopathologically in the high dose group, and that none were examined in lower dose groups. Also, concerning the acute and subchronic neurotoxicity studies mentioned, I would cite my memorandum of November 20, 1997 to Clark Swentzel as detailing comments I might otherwise offer here.

2. Determination of Sensitivity
No further comments on the developmental and reproduction studies.

VII **Data Gap(s)**

Roman numerals go from III to VII in the HIDR.

From my perspective, the following are data gaps:

1. Carcinogenicity Study in B6C3F1 Mice (MRID 43407201) :
Pathology Working Group assessment for liver tumors;
Histopathology assessment of nasal tissues.

2. Combined Chronic Toxicity/Carcinogenicity F344 Rat Study (MRID 43942901) : Pathology evaluation/reevaluations of various tissues.

3. Subchronic Inhalation Study in Sprague-Dawley Rat (MRID 43266601) : resolution of no NOEL for nasal tissue histopathology, which was severe at the lowest dose and present in essentially all rats of both sexes; recommend a new and longer term study to address the absence of a NOEL and potential carcinogenicity by the inhalational route.

4. Developmental Toxicity Study in the Rabbit (MRID 152569) : submission of Appendix III followed by statistical treatment of the individual data to affirm the NOEL for body weight effects in dams particularly over days 6-12 of gestation.

5. Acute Neurotoxicity Study in the F344 Rat (MRID 43146701): submission of selected retinal tissue slides as called for in the DER.

6. Subchronic Neurotoxicity Study in the F344 Rat (MRID 43269501) : submission of a guideline behavioral test yet to be specified.

7. Three-month cholinesterase assay in the rat to determine a definitive LOEL/NOEL for malathion.
Brian Dementi, Ph.D, Toxicologist/HED

ATTACHMENT 7:Letter from B. Dementi - January 15, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

January 15, 1998

Re: Additional information concerninig the malathion acute neurotoxicity study.

One of the issues before the Hazard ID Committee in assessing the malathion data base is that of one incident of bilateral retinal rosette among five male rats in the high dose group in the acute neurotoxicity study. You will recall that the DER for that study calls for submission of selected histopathology slides for independent characterization of retinal rosette. I will not reiterate here that which has already been presented in earlier documents, but would like to introduce some additional information that may have some significance in the deliberative process.

Various published works indicate that the terms retinal rosette, retinal fold and retinal detachment may apply to the same or very similar anatomic or pathologic condition, all of which seem to involve a separation and folding of certain layers of the retina. This in itself is a complicated subject, and there may be varying terminologies for this phenomenon. [Tansley (1933); Lai and Rana (1985); Rubin (1874); Kuno et al (1991)] Furthermore, retinal rosettes are said to be rare in rats and are generally considered to be developmental effects, not occurring spontaneously in adult animals. According to Tansley (1933), an older but excellent reference, it is claimed that there is normally a difference in tension between the outer and inner layers of the ratina explained as follows: "That there is normally some difference in tension between the outer and inner parts, even in the adult retina, can be shown by removing it from the eye. If the tissues are still living, it will be found that the retina always curles up into a roll so that the rod and cone layer is on the inside." Further along the author says, "Under the conditions described in this paper there certainly seems to be a definite relation between the maintenance of a normal intra-ocular pressure and the appearance of retinal rosettes. In all the cases with which we have been dealing here the fact of a lowering or an absence of intra-ocular pressure also involves the dissociation of the retina from the wall of the eye to which it is normally attached during development" (p. 335) It is as if intra-ocular pressure helps sustain, physically, the retina in its normal contours. This particular publication involved studies on postnatal eye development in rats, but the implication is that even in the adult eye, intra-ocular pressure may be critical to the maintanance of retinal form.

Since organophosphate cholinesterase inhibitors have a medicinal use in the treatment of glaucoma, via reducing intra-ocular pressure, I decided to examine precautionary labeling on such medication. In the case of Ayerst Laboratories' Phospholine Iodide (echothiophate iodide for ophthalmic solution), an organophosphorothioate cholinesterase inhibitor, under Adverse Reactions, the first mentioned reads as follows, "Although the relationship, if any, of retinal detachment to the administration of Phospholine Iodide has not been established, retinal detachment has been reported in a few cases during the use of Phospholine Iodide in adult patients without a previous history of this disorder." Further, under Precautions, the seventh reads "Phospholine Iodide (echothiophate iodide) should be used with great caution, if at all, where there is a prior history of retinal detachment." It is also noteworthy that the echothiophate insert indicates that echothiophate potentiates other cholinesterase inhibitors such as organophosphate and carbamate insecticides, and that patients undergoing systemic anticholinesterase treatment should be warned of the possible additive effects. A copy of the insert where these quotes may be found is appended.

In summary, this added information indicates that: 1) retinal rosettes, retinal folds and retinal detachments

(even microretinal detachments) may be different terms for a common underlying effect; 2) maintanance of intra-ocular pressure may play an essential role in preserving retinal structure; 3) substantial declines in intra-ocular pressure could in principle elicit retinal detachment and/or scrolling of the retina; 4) organophosphate medicinals used to control intra-ocular pressure in the treatment of glaucoma, presumably when used with precise dosing under the care of a physician, have as an associated precaution retinal detachment; 5) malathion as evaluated in the acute neurotoxicity at single very high doses via oral gavage, in fact could have elicited a precipitous decline in intra-ocular pressure resulting in the retinal anomaly; 6) malaoxon, the active metabolite of malathion, like echothiophate is an organophosphorothioate. So whatever the mechanism of the possible association between treatment with echothiophate and retinal detachments in humans might be, that mechanism could in principle operate in the acute neurotoxicity study where very large doses were used.

Brian Dementi, Ph.D.
Toxicologist, HED

cc Jess Rowland

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ATTACHMENT 8: Letter from B. Dementi - February 10, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

February 10, 1998

In drafting the toxicology chapter of the Registration Eligibility Document (RED) for malathion and attendant closer evaluation of the Hazard ID Committee report for the November 6, 1997 meeting to consider malathion, there is yet another issue of concern that I believe merits resolution.

Under the topic of Reproductive Toxicity (pp. 15-16) of the Committee report, the following paragraph is found. "Although the offspring NOEL (131 mg/kg/day in males and 153 mg/kg/day in females) was lower than the parental systemic NOEL (394 mg/kg/day in males and 451 mg/kg/day in females), the Committee determined that this was not a true indication of increased sensitivity of offspring because: (I) pup body weight decrements were primarily observed at postnatal day 21; (ii) during that period (i.e., later portion of lactation), young rats consume approximately twice the diet per unit body weight as an adult rat consumes (i.e. 1 ppm in the diet of a young rat is approximately 0.1 mg/kg/day whereas in older rats, this ppm level is equal to 0.05 mg/kg/day) and (iii) the estimation of the test substance intake in pre-weaning animals is likely to be more than double the adult intake because of the availability of the test material both via the milk (lactation) and food, particularly after the mid point of lactation." While there is much that may be viewed as generally true in this statement, my concerns have to do with the reliability one can place in these arguments in this particular case, lacking definitive data, to conclude that offspring were no more sensitive than adults.

Although it is true that weight decrements were primarily observed at postnatal day 21, pup body weight decrements were statistically significant on days 7, 14 and 21 for the F2B generation at the penultimate dose level, which the study report itself concluded to be treatment related. No record is made in studies such as this of pup food consumption, so it is very presumptive in the particular case at hand to draw conclusions about what pups may or may not have consumed in the control and various dose groups. Generalities regarding relative food consumption of pups versus adults cannot be reasonably used to reach definitive conclusions pertaining to how much test material various dose groups may have been exposed to via the diet. Furthermore, there is no data in this study to show the presence or absence of malathion in the milk. There is an incumbency to have, or provide, data showing not only the presence of malathion in the milk, but how much is there, before concluding that malathion ingestion via the milk contributed in a sufficiently meaningful way to total intake and thus to support the argument that pup consumption on a body weight basis exceeded that of adults in this study. Hence, the reasoning used to dismiss the finding in this study of greater sensitivity of offspring are speculative, and certainly not of the definitive character required to refute the positive evidence that pups were in fact shown more sensitive than adults.

This particular evidence of increased sensitivity of offspring takes on peculiar importance in the assessment of malathion by the Hazard ID Committee in relation to the question of whether to remove the 10X factor required under FQPA for infants and children. The Committee concluded that the 10X factor should be removed in part because: " A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults." (p. 18) The Food Quality Protection Act (1996) requires use of an extra 10-fold safety factor in addition to the traditional 100-fold safety factor, unless, on the basis of reliable (emphasis added) data, a different level is determined to be safe for children. It is my understanding

that the intent of Congress, as reflected in FQPA, was to afford additional protection for infants and children unless and until it can be reliably shown that the 10X or a lesser added factor is not needed. The burden of proof rests with the Agency to show via the absence of any evidence of increased sensitivity of offspring, and in the face of a complete data base, that the added factor is not necessary. As I endeavored to persuade the Committee in my December 17, 1997 comments addressed to Jess Rowland, developmental and reproductive toxicity studies are inherently weak to detect the more subtle effects of cholinesterase inhibition, indeed such studies do not even incorporate cholinesterase assessments, therefore there is enhanced reason to rely on parameters such as body weight, crude as they may be, as evidence of effects of a test material. If cholinesterease data in offspring and that versus adults were available in this study, less reliance would need to be placed on the body weight data. But given the situtation as it exists, I consider the reasoning used by the Committee to dismiss evidence of enhanced sensitivity in offspring in the two generation reproduction study to violate the intent of Congress to the end that the 10X factor be discounted only on the basis of reliable data.

Brian Dementi, Ph.D.
Toxicologist, HED

cc Jess Rowland

ATTACHMENT 9: Letter from B. Dementi - March 10, 1998

Jess Rowland, Secretary
Hazard ID Committee

March 10, 1998

This is an addendum to my December 17, 1997 comments to you on the Hazard ID Committee report for the November 6, 1997 meeting on malathion. My comments here pertain to the subchronic inhalation study. I recently requested from the registrant's representative a copy of the range-finding inhalation study. The study is entitled "A 2-Week Toxicity Study of Aerosolized Malathion Administered by Whole-body Inhalation Exposure to the Albino Rat" completed on July 20, 1993. Concentrations evaluated in this study were 0, 0.56, 1.58 and 4.23 mg/L, as contrasted with those employed in the full subchronic study of 0, 0.1, 0.45 and 2.01mg/L. After two weeks of treatment, with respect to upper respiratory findings, the Summary of the study claims that histological findings on the nasal and laryngeal mucosa were observed in most low dose animals and in the majority of the mid and high dose animals. "These findings included a slight to mild loss of goblet cells and similar hyperplasia in the nasal respiratory epithelium, slight leucocyte exocytosis in the nasal squamous and respiratory epithelium and slight to mild epithelial hyperplasia of the laryngeal mucosa." (p. 10) The fact that there was no NOEL for nasal and laryngeal effects after only two weeks of exposure demonstrates a much earlier onset of the nasal effects than could be determined from the subchronic inhalation study with malathion or the chronic feeding studies with malathion and malaoxon, where similar nasal and laryngeal effects were observed.

These histopathologic findings, without a NOEL, in this range-finding study after only two weeks of exposure, taken together with similar findings in the other longer term studies, serve to reinforce my opinion that another inhalation study is needed to identify a NOEL, and to determine the time of onset and ultimate course for nasal and laryngeal effects. Again, I consider inadequate the Hazard ID Committee's decision to employ a UF of 3 to compensate for the absent NOEL for this effect in the subchronic inhalation study. Your February 1997 Guidance Document for the Toxicology Endpoint Selection Process claims that "However, a LOEL may be used if a NOEL is not established in the critical study, when severity of the effects observed at this dose is of negligible concern for human risk, or when there is a data gap. Therefore, when a LOEL is identified for risk assessment, additional modifying factors (range of 3 to 10) may be used in addition to the total Uncertainty Factorof 100 (i.e., 10 for intra- and 10 for inter-species variation)." (p. 12) In response to this, I cannot accept the premise that the severity of the nasal and laryngeal tissue effects are to be viewed as of such "negligible" concern for human risk as to justify use of a modifying factor as explained in your paper. Furthermore, if the committee were inclined on employing a modifying factor of between 3 and 10, what reasoning was invoked to support choosing the low factor? Please be reminded that at the Cancer Assessment Review Committee meeting of last September-October these nasal tissue findings in the chronic feeding studies were considered of sufficient concern as to require additional nasal histopathology in the malathion rat and mouse studies.

Brian Dementi, Ph.D.
Toxicologist/HED

ATTACHMENT 10: Letter from B. Dementi - March 16, 1998

Jess Rowland, Secretary
Hazard ID Committee

March 16, 1998

This is a further addendum to my December 17, 1997 comments to you on the Hazard ID Committee report for the November 6, 1997 meeting on malathion. My comments concern a 2-week range-finding inhalation study which I mentioned to you in my March 10, 1998 memorandum. In that earlier communication I commented on histopathology findings in nasal and laryngeal tissues. In this case I would like to advise that in the range-finding study involving test concentrations of 0, 0.56, 1.58 and 4.23 mg/L, a NOEL was not identified for erythrocyte cholinesterase inhibition in either sex, or for plasma or brain cholinesterase inhibition in females. In males, the NOELs for plasma and brain cholinesterase inhibition were 0.56 mg/L and 1.58 mg/L, respectively. The number of rats under test in this study was but 5/sex/group (in certain groups the number composing mean values for cholinesterase activity were less than five due to various reasons given in tables of individual data), and there was no statistical treatment of the cholinesterase data in the study report as submitted. So the reported findings cannot be considered definitive, as is often characteristic of range-finding studies. However, it should be noted that in terms of percent enzyme inhibition relative to controls, after two-weeks of exposure in this range-finding study, cholinesterase inhibition (plasma, erythrocyte and brain) is reasonably complementary with that of the full subchronic inhalation study after 90-days of treatment. To the extent that the data may be considered reliable, there is little evidence of a cumulative effect of malathion over 13 weeks as opposed to 2 weeks. The two studies taken together (see attached table) yield reasonably consistent dose-reaponse data across the overall dose range of 0.1 to 4.23 mg/L, with the data indicating that erythrocyte cholinesterase is virtually equally responsive in both sexes, but that females are more remarkably affected in terms of plasma and brain cholinestrerase inhibition. The range-finding data also tends to strengthen the conclusion in the subchronic study that there is no NOEL for plasma cholinesterase inhibition in females and possibly for erythrocyte cholinesterase in both sexes. Overall, the data indicate females to be the more sensitive gender.

Brian Dementi, Ph.D.
Toxicologist/HED

Comparative Cholinesterase Inhibition in 2-Week Range-Finding and 90-Day Subchronic Inhalation Studies in Sprague-Dawley Rats

	Atmospheric Concentrations (mg/L)					
	0.1	0.45	**0.56**	**1.58**	2.01	**4.23**
Enzyme Inhibition,%						
Plasma Cholinesterase						
Males:	2	7	7	20	18	50
Females:	16	30	49	71	70	84
Erythrocyte Cholinesterase						
Males:	9	22	18	33	43	58
Females:	11	27	26	39	44	53
Brain Cholinesterase						
Males:	5	3	0	4	17	36
Females:	4	8	12	18	41	59

Notes: Bold print atmospheric concentrations from 2-week range-finding inhalation study (5 rats/sex/group); Normal print concentrations from subchronic (90-day) inhalation study at term (15 rats/sex/group).

ATTACHMENT 11: Letter from B.Dementi - March 20, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

March 20, 1998

Re: Acceptability of the malathion chronic dog study by the Hazard ID Committee; recommendation for additional testing of cholinesterase inhibition in dogs.

The following comments are intended to summarize the historical record regarding the status of the acceptability of the malathion one-year chronic dog study (MRID 40188501), and to present rationale in support of additional testing for cholinesterase inhibition in the dog.

The October 5, 1987 Data Evaluation Record (DER) concluded the chronic dog study to be *Supplementary,* attributable to the lack of NOELs on several toxicology end points. The October 7, 1987 covering memorandum addressed to the Product Manager called the study *Supplementary* and claimed it would not serve to satisfy guideline requirements. When the study was taken under advisement by the TOX-SAC (report date: September 11, 1997), that committee concluded the study to be *Unacceptable.* In accordance with that conclusion, the DER was subsequently revised in preparation for the November 6 Hazard ID Committee presentation. The revised DER claimed that: "This study is **NOT ACCEPTABLE** (Supplementary) and **DOES NOT SATISFY** guideline 83-1 for a chronic toxicity study in dogs because NOELs were not established for inhibition of cholinesterase activity for plasma and erythrocytes in either males or females." I interpret this to mean that among the several NOELs cited in the original DER, the TOX-SAC narrowed the findings of concern down to cholinesterase inhibition as the basis for claiming the study to be *Unacceptable.* This was the assessment prior to the Hazard ID Committee meeting.

Having examined the December 17, 1997 report for the Hazard ID Committee meeting, I find no specific reference to any deliberations regarding the status of acceptability of the study, nor any statement to the effect that the committee reversed the conclusions of the TOX-SAC. In recent consultation with the toxicologist who presented the data base to the Hazard ID Committee, he speaks of having no recollection of raising the issue; nor do I recall its being mentioned, though perhaps it was so mentioned in passing. The December 17 report does say there are no data gaps, but there is the question as to whether that statement refers to the entire data base, or more narrowly to those studies that would address relative sensitivities of developing and young individuals to adults for purposes of deciding the need for the FQPA imposed 10X safety factor to protect infants and children. To the extent that the "no data gaps" statement refers to the entire data base, we know that is incorrect because of the unresolved malathion carcinogenicity issues identified at the September-October 1997 Cancer Assessment Review Committee meeting. I believe in order to set the record straight, the report of the Hazard ID Committee meeting should convey in unambiguous terms that the committee did not concur with the TOX-SAC, if that is indeed what transpired..

As relevant to this question, your committee should be advised that a memorandum was written on March 26, 1990 by B. Dementi to P. Fenner-Crisp, then director of HED, conveying the results of a March 1, 1990 Cholinesterase Peer Review Committee meeting in which the status of the acceptability of the dog study was reviewed. That committee concluded that the study would satisfy the section 83-1 data requirement. (Attachment 1) I doubt that this memorandum was among documents reviewed by either the TOX-SAC or the Hazard ID Committee. Presumably the results of that 1990 peer review were communicated to the Product

Manager, and thence to the registrant. The March 27, 1997 malathion oneliners lists the study as Supplementary without offering any qualification.

Given this background, I consider it necessary to provide additional perspective on this subject. In April of 1992, I drafted a memorandum addressed to the Product Manager recommending further study in the dog. That draft memorandum was signed by me on April 16, 1992. (Attachment 2). The reasoning for that recommendation, as set forth in the memorandum, was in part to help determine whether the dog or the rat should be selected as the preferred surrogate species for humans in ocular effects testing [see Exhibit I (p. 5) of my November 10, 1997 letter to you]. Ocular effects of organophosphates had become an important topic at that time, and remains so today. The April memo also claimed the work was needed to identify the NOEL for cholinesterase inhibition in the dog, as it was not achieved in the subject study. I must stand behind that recommendation today.

Supporting this recommendation, it may now be said, is the responsibility to obtain **reliable** (a word judiciously employed under FQPA) assessments of cholinesterase inhibition in the dog. I would note the following. In the February 1988 Malathion Registration Standard (OMB Control No. 2070-0057), the guideline requirement for a 90-Day (Subchronic) Feeding Study in the dog was waived:"...since requirments for chronic rodent and non-rodent toxicity studies have been imposed." (p. 123) Since the subchronic study was waived, there is to that extent, in my judgment, a greater burden to have in place a fully acceptable chronic study. The subchronic study would have been a feeding study which could have helped addressed the question of whether the method of dosing (oral capsule) in the chronic study compromised the expression of cholinesterase inhibition, and perhaps whether the dog is as refractory to malathion induced cholinesterase inhibition, as some seem willing to accept. Indeed, in my opinion the obtaining of an acceptable chronic dog study is implicit in the waiver of the subchronic study. I recently examined the malathion one-liners for dog feeding studies, and found none. So other than the one chronic study, there is a paucity of relevant data on dogs.

There are yet additional reasons why **reliable** cholinesterase data should be in place with respect to the dog: 1) The FIFRA guidelines intend that acceptable data be in place on multiple species since animal models are used as surrogates for human responses. It should be viewed as especially important in this particular case with malathion, because **reliable** data on the dog is not available, in the face of a remarkable contrast between the sensitivities of human and rat, i.e. definitive data on a third species is indicated; 2) As is true in the case of humans, the dog lacks carboxylesterase in the plasma [see Exhibit 1 (pp. 3-5) in my November 10, 1997 to you], which in principle should render the dog (like the human) more susceptible than the rat to the cholinesterase inhibiting effects of malathion; 3) I recently compared the dog and rat studies for a few organophosphates in the Caswell file and found the dog to be very responsive, exhibiting no such remarkable differences versus the rat as is evident in the malathion case; 4) Since the dog study was reviewed, serious questions have arisen within HED as to the adequacy of cholinesterase methodology employed in data submissions in general. I have participated in workshops on cholinesterase methodology. In my view this is a serious matter with respect to the degree of **reliance** to be accorded data submissions, particularly those such as the malathion chronic dog study where there is considerable puzzlement over the apparent lack of responsiveness and no adequate explanation. Further, I remain uncertain at this time as to the final outcome of in-house assessments pertaining to the grander question of the adequacy of cholinesterase methodology, and what may have been recommended in more recent times that would assure proper assessment of cholinesterase inhibition. In retrospect, I now believe the 1990 Cholinesterase Committee erred in assuming that because the dog yielded a weak response in the chronic study that the NOEL, once properly determined, would be far above that in the rat and human. Such a position accepts uncritically that cholinesterase methodology employed in the study was satisfactory. Furthermore that view neglects to recognizer that the response could may be entirely different in a feeding study, such as the one waived.

The bottom line to all this is that there exists a data gap for cholinesterase inhibition in the dog. In my view, an additional malathion study should be required to allay concerns over the questionable data now in place. This is a particularly important requirement since the RfD is based on cholinesterase inhibition, and the Hazard ID Committee has shifted the defining study from that of Moeller and Rider (1962) in humans to that of the recently submitted chronic toxicity/carcinogenicity study in the rat, which study has an anomaly at the lowest dose for erythrocyte cholinesterase inhibition in females, as I have noted in my December 17, 1997 comments to Jess Rowland, and presented more fully in my November 10, 1997 letter to you. I consider it unfortunate if the registrant has been advised that no additional work in the dog is necessary. However, perspectives have changed since the 1990 peer review, and the public interest is of far greater moment than the additional effort this requirement would entail.

Sincerely,

Brian Dementi, Ph.D.
Toxicologist/HED

cc Jess Rowland

ATTACHMENT 12: Letter from B. Dementi - July 27, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

July 27, 1998

Re: Malathion External Peer Reviews

As you know, following the November 6, 1997 Hazard ID Committee meeting on malathion, I drafted a number of letters in response to the minutes, directed either to you or Jess Rowland. As an approach to addressing my questions, the Office elected to invite comments from external experts in toxicology. In preparing for the external peer review, I drafted a set of questions, numbered I-VIII, accompanied by pertinent reference materials, which were provided to the reviewers via OPP's external peer review coordinator, Dr. Hank Spencer. OPP introduced, preliminary to my set of specific questions, a "Charge to the Reviewers" which called for quality assessments of the various DERs in general, and whether the appropriate uncertainty factor was used for the RfD.

The external reviewers, Drs. Michael Dourson, Rolf Hartung and Walter Decker, have now provided their responses. Their letters are expected to be included in the package of documents to be considered by the Hazard ID Committee at its August 18 meeting. I would like to request that you provide Committee members a copy of the entire package, including all referenced materials, that was available to the external reviewers. This represents considerably more information than was presented on November 6. I would hope the Committee might have adequate time, possibly a little more than usual, to study the package. I would be available in the interim to respond to any questions or comments anyone wishes to pose.

In an effort to compare and interpret the reviewers' responses to my questions, I have consolidated, in the format of the same questions, abbreviated conclusions of each reviewer under each question, in order to view juxtaposed the responses to assess the level of concurrence and the extent to which they, collectively, have helped me and hopefully others in understanding the facts before us. The abbreviated conclusions represent my best judgement of what they communicated as gleaned from their more detailed responses. So I would urge Committee members to confirm whether my interpretations are appropriate.

Also, under each question, I have included comments which represent my effort to estimate not only how well the reviewers agree, but whether and to what extent they have guided me in addressing my questions. This *Consolidation of External Peer Reviewers' Comments on Malathion Non-Cancer Issues* dated July 27, 1998, written by me, is appended to this memorandum.

Brian Dementi, Ph.D. DABT
Toxicologist
Toxicology Branch I/HED

CONSOLIDATION of EXTERNAL PEER REVIEWER'S COMMENTS on MALATHION NON-CANCER ISSUES. by BRIAN DEMENTI JULY 21, 1998

I Hazard Identification/Acute Oral (One-Day)

Supporting documentation: DER #s 5, 6, 7, 9 and 19; References: A (pp. 3-5), B (pp.1-4), C, D, E , V and BB (pp. 12-14; 20-22)

Question 1): **Do the rabbit developmenal toxicity and developmental range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicologic consequence in either the maternal or the developing organisms?**

Dr. Dourson: No.

Dr. Hartung: No.

Dr. Decker: No.

Comments: The external reviewers do not accept that a single dose as high as 50 mg/kg would be without toxicologic effect in maternal or developing organisms based on the rabbit developmental toxicity studies.

Question 2): **Does data on maternal body weight and body weight gain now available in Appendix III of the rabbit developmental toxicity study alter the assignment of the LOEL/NOEL for the study, and does it influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxicologic effect?**

Dr. Dourson: No.

Dr. Hartung: No.

Dr. Decker: No.

Comments: The external reviewers agree that data in Appendix III would not influence the conclusion. We should note that data in this appendix has not been analyzed, statistically, in HED.

Question 3): **As presented in a published work in the open literature, single intraperitoneal doses as low as 50 mg/kg in the rat reportedly elicited a clear effect on avoidance performance while cholinesterase inhibition (erythrocyte) was observed at 100 mg/kg. Plasma and brain cholinesterases were also inhibited at 150 mg/kg. Cholinesterase inhibition and decrements in behavior were all very significant though transient effects: a) What level of confidence should be accorded this study?; b) What is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg can serve as the endpoint for acute dietary (one-day) risk assessment?; c) Is the data available in the developmental toxicity studies sufficiently reliable to discount the 10X safety factor required under FQPA?**

Dr. Dourson: Says the study has advantage of testing a relevant effect. Route of exposure is an issue. "I am not satisfied that potential risks to humans is addressed with the data available in this review package. But more data are probably available to further address this question. A discussion of uncertainty factors for potential data base gaps should be postponed pending the review of these additional data." (p. 4)

Dr. Hartung: Says behavioral effects that have a degree of correspondance with cholinesterase inhibition are to be expected, but there is no requirement that dose response curves for both to coincide. Intraperitoneal route is of questionable surrogacy for realistic exposures. Says data does not support deletion of the 10X factor.

Dr. Decker: Accord low level of confidence to the study because i.p. cannot directly compare to real exposures. Says cannot dismiss the 10X factor

Comments: The external reviewers consider the study to be of value in that it assesses relevant effects, and supports a degree of correspondance between cholinesterase inhibition and behavioral effects, but all appear to agree that data from developmental toxicity studies, and perhaps the entire malathion data base, does not support deletion of the 10X safety factor imposed by FQPA. My principal reason for citing Kurtz (Ref. D) was to illustrate that a single dose at 50 mg/kg can elicit a remarkable response. Furthermore, the study shows that at doses extending below those inhibiting cholinesterase, a behavioral effect has been observed, even if the route of administration differs from that of normal human exposure. None of the reviewers question the quality of the study, or the validity of the findings.

II Determination of Susceptability, Reproductive Toxicity

Supporting documentation: DER: # 5; References: A (pp. 15-16), B (pp. 3-4), F, G and BB (pp. 12-14; 16-17; 20-22)

Question 1) **Can the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation reproduction study in the Sprague-Dawley rat be dismissed as "...not a true indication of increased sensitivity of offspring..." for the reasons stated in the Hazard ID Committee report?**

Dr. Dourson: Yes, to the extent that the dose in offspring is not derived from actual assessment of food intake.

Dr. Hartung: Yes, but expresses the view that neonates must be shown to be less sensitive than adults (not equal to) before the FQPA 10X safety factor can be deleted.

Dr. Decker: No, "because some toxic effects have been reported."

Comments: Two reviewers say yes (with qualifying remarks) and one says no. I had hoped the reviewers would say something specific about views expressed in Ref. F, supported by data in Ref. G (selected pages from the study report). The point is that an effect on pup body weight occurred at a dose below that which similarly affected dam body weight. The effect on pups was dismissed by the Hazard ID Committee as evidence of greater sensitivity of pups for reasons which in my view were unsubstantiated, i.e. no proof of the presence of malathion in the milk, nor any evidence of how much food pups may have consumed under circumstances wherein malathion in the diet may have influenced food intake. It may not have been clear to the external reviewers that the presence (let alone the amount) of malathion in the milk has not been shown by analysis. It should also be noted that while pup body weight changes were seen during lactation days 7

(where pups rely essentially exclusively on milk), 14 and 21 in the 5000 ppm dose group (the NOEL for dam body weight change in the study at large), dam body weight changes were not apparent during the lactation period even at the top dose of 7500 ppm. Hence, during lactation pup NOEL/LOEL = 1700/5000 ppm, while dam NOEL ≥ 7500 ppm (HDT). Pope and Chakraborti (1992) (Ref. E) say that young mammals are remarkably more sensitive than adults to numerous organophosphates. Hence, the burden is not light to justify dismissing evidence of a more selective effect in pups due to exposure to this particular OP.

Question 2) **In the absence of assessments of cholinesterase inhibition and behavioral effects testing in adult and young animals in reproduction studies, can the data obtained in the FIFRA guideline study be considered adequate to address the question of whether young or mature animals are the more sensitive to malathion?**

Dr. Dourson: No.

Dr. Hartung: Seems to say no since the data in question do not exist. Though at this point he does not actually affirm the critical importance of the data in question, he attests to the importance elsewhere in the document. For example, in defending the use of the human cholinesterase study, Moeller and Rider, he says: "....it addresses a diagnostic end-point that is known to be mechanistically related to the toxicity of OPs." (p. 8); and "Changes in some behavioral parameters that have a degree of correspondance to acetylcholinesterase, in particular to brain cholinesterase, would be expected." (p. 5)

Dr. Decker: No. Says more behavioral (learning) tests should be performed. FIFRA Guidelines need updating.

Comments: The external reviewers appear to agree in saying no to this question, i.e. data in the 2-generation reproduction study are not adequate to address the question of relative sensitivity of young versus mature animals.

Question 3) **Does this two-generation reproduction study provide the reliable evidence of no increased sensitivity in pups when compared to adults, as required under FQPA, to discount the 10X safety factor imposed by FQPA as additional protection for infants and children?**

Dr. Dourson: Suggests 3X as opposed to 10X safety factor. Although, he acknowledges 10X may still be useful as a management tool.

Dr. Hartung: No. Expresses view that the study shows no clear evidence of less sensitivity of offspring, which he considers essential.

Dr. Decker: No. "....evidence seems quite thin." (p. 5)

Comments: The weight of opinion is that the 10X safety factor under FQPA cannot be dismissed.

III Hazard Identification/Chronic Dietary (RfD)

Supporting documentation: DERs: #s 1 and 10; References: A (pp. 5-6), B (pp. 4-5), H, I, N (p. 16), R and Y.

Question 1) **Given the evidence of a post 3 months recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, can 50 ppm be concluded to have been a NOEL for the first three months of testing?**

Dr. Dourson: Yes, but recommends an additional 3-fold uncertainty factor be applied to the NOEL in the rat in establishing the new RfD, as indicated in question 5.

Dr. Hartung: No.

Dr. Decker: No.

Comments: Dr. Dourson says yes to this question, but it is not clear what his opinion would be in the event an additional uncertainty factor were not used with the rat data as he proposes. The other two reviewers agree that it cannot be said that 50 ppm was a NOEL in view of the findings in the background papers. Elsewhere in their comments, Dr. Hartung says: "I find the discussion regarding the selection of plasma cholinesterase inhibition for the determination of the RfD to be simplistic and superficial." (p. 3) Dr. Decker says with regard to the question of whether the human or rat data should be used for establishing the RfD: "I recommend that Dr. Dementi's suggestions be actively pursued, that is more studies are needed to fill in data gaps." (p. 4) Dr. Decker thus acknowledges data gaps. He also says: "I am not aware of supporting studies which shore up the use of the principal study for the RfD." (p. 4) It is reasonable therefore to conclude that a consesus exists that the study does not satisfactorily identify a NOEL for cholinesterase inhibition. It should be noted that the registrant was advised before conducting the chronic toxicity/carcinogenicity study in the rat that 100 ppm would be expected to be an effect level for cholinesterase inhibition (Ref. I) Three months is an important time period, as within this time frame important adjustments to the treatment may occur.

Question 2) **Alternatively, do these findings suggest flawed cholinesterase methodology, and if so, what corrective measure could be pursued?**

Dr. Dourson: No comment on cholinesterase methodology.

Dr. Hartung: Says requires analysis of detailed cholinesterase methodology.

Dr. Decker: Says this is a possibility, and if so, concern extends to all OP pesticides.

Comments: This question was posed primarily because erythrocyte cholinesterase was clearly inhibited in females at the 100 ppm and 500 ppm dose level after three months of dosing, but not at 50 ppm or 500 ppm at six months. These contrasting findings at 500 ppm cloud the interpretation as to whether 50 ppm would have been an effect at three months had it been tested. In the views of the external reviewers, it would appear to be an outstanding question that requires resolution. Perhaps results of OPP's workshops on cholinesterase methodology could help resolve this question.

Question 3) **Should 4 mg/kg/day, the NOEL for plasma cholinesterase inhibition in males, be supported as a replacement for human data previously relied upon in establishing the RfD, or should additional testing be required in the rat to identify a NOEL for cholinesterase inhibition, particularly in females?**

Dr. Dourson: Yes to the first part of question. Says additional testing not needed. Suggests benchmark dose analysis in event some scientists wish to pursue whether 50 ppm is a NOEL in females. Notes that 50 ppm was a NOAEL in the 13-week neurotoxicity study. However, recommends additional 3-fold uncertainty factor as indicated in Question 5.

Dr. Hartung: No to the first part of question, and is critical about replacing human data with animal data.

Dr. Decker: No to the first part of question. Recommends additional testing to identify NOEL in rats of both sexes.

Comments: Same as those under question # 1. In addition I should reference my concerns about placing reliance upon the NOEL for cholinesterase inhibition in the 13-week neurotoxicity study as expressed in Ref. B (pp. 4-5).

Question 4) **Given that an explanation exists for a greater sensitivity of humans than rats with respect to cholinesterase inhibition from malathion exposure (i.e. the lack of carboxylesterase in human plasma) should a 10X safety factor applied to the rat data to allow for "uncertainties" in interspecies variability be considered adequate if the rat data is to be used in deriving the RfD?**

Dr. Dourson: Yes, but advocates an additional 3-fold uncertainty factor for other reasons as indicated in question 5.

Dr. Hartung: No

Dr. Decker: No, but would be acceptable with enhanced testing in the rat.

Comments: The reviewers' comments are important in underscoring the fact that the data base is inadequate as it stands in establishing an RfD. Actually, in posing this question, I was seeking the reviewers' opinions as to whether the concept of using a 10-fold safety factor intended to account for uncertainties in interspecies variability is adequate in the face of known differences in sensitivity. Stated differently, should corrections to accomodate know differences, which may even exceed 10-fold, first be introduced, followed by the 10-fold factor to address the unknown species differences in susceptability? (Ref. I) It is not clear to me that this particular philosophical question was recognized or responded to, but remains a question for the Hazard ID Committee.

Question 5) **Further, given that the RfD based on human data (0.023 mg/kg/day) is lower than that derived from the rat data (0.040 mg/kg/day) and that an explanation exists for a greater sensitivity for humans, should the RfD based on human data be retained?**

Dr. Dourson: No, but advocates an additional 3-fold uncertainty factor to account for deficiencies in the data base, principally because the critical effect (cholinesterase inhibition) was not monitored in the 2-generation reproduction study in a potentially sensitive subgroup (i.e. young rats), which he characterizes as a data gap (p. 3). Also, suggests an added uncertainty factor of unspecified magnitude, probably less than 3 in his view, for the RfD based on the human study, should it be retained, since females (women) were not tested.

Dr. Hartung: Yes.

Dr. Decker: Yes.

Comments: Given that Drs. Hartung and Decker say, emphatically, the human study should be retained, and Dr Dourson does not provided an unqualified differing opinion, a consesus exists that the human study should be retained. If it is to be retained, an added safety factor should be considered based upon Dr. Dourson's comments..

Question 6) **Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?**

Dr. Dourson: Does not answer the question as such, but acknowledges in Question IV, # 5 recognition the study does not test toxicity in young rats, and, hence, lacks surrogacy for infants and children. He asserts that the FQPA safety factor should not be considered in a discussion of science. He discusses his interpretation of the FQPA 10X factor as a safety factor for use in risk management toward the protection of infants and children, as opposed to that of an uncertainty factor.

Dr. Hartung: No, since the available information does not support the hypothesis that neonates are less sensitive than adults (see his p. 6)

Dr. Decker: No.

Comments: In disagreeing with the context of the use of the 10X safety factor, Dr. Dourson in my view did not respond with an opinion as to whether this study in any way supports discounting imposing the factor. Drs. Hartung and Decker say no. It would appear reasonable to conclude the reviewers feel the study does not provide any support for discounting use of the safety factor.

IV Subchronic Inhalation Study

Supporting documentation: DERs: #s 1, 2 and 13; References: A (pp. 9-11), B (pp. 5-6), J, N (p. 12) and O. ***(Note to Hazard ID Committee: please also see Ref. CC. This reference was in the package submitted to the external reviewers, but was not listed here among supporting documents for this question.)***

Question 1) **Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngal degeneration/hyperplasia supportable?**

Dr. Dourson: No. Advocates use of 10X rather than 3X uncertainty factor.

Dr. Hartung: No. Questions inhalation test procedure (whole body). Says finetuning (i.e., interpreted to mean use of 3X, or other factor) cannot accommodate gross deficiencies.

Dr. Decker: Says does not understand derivation of 3X uncertainty factor.

Comments: Given the inability for Dr. Decker to respond, taken in concert with the negative responses of Drs Dourson and Hartung, the consesus of the external reviewers is that use of a mere 3X uncertainty factor is inadequate.

Question 2) **A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not identify NOELs for cholinesterase inhibition or histopathology findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to envoke an uncertainty factor for acute risk assessment (i.e. 1-7 days) on the basis of cumulative effects?**

Dr. Dourson: Yes (implied). Presents the argument that comparative findings in the 2-week and 90-day studies do not support a very remarkable cumulative response, and thereby, perhaps unwittingly, dismantles the Hazard ID Committee's principal argument for not invoking the uncertainty factor in the case of short-term exposures.

Dr. Hartung: No. Same comment as in question 1

Dr. Decker: Says a rangefinding study should not be used to decide, since such studies do not provide reliable information.

Comments: Given the nature of responses from all three reviewers, I believe the question was not particularly clear. The Hazard ID Committee advocated a 3X uncertainty factor for the intermediate and long-term, but not for short-term(1-7 days) exposure risk assessments. The decision for not invoking the factor for the short-term exposures was predicated on the assumption that the end points in question identified in the 90-day inhalation study were cumulative in nature, and would not likely occur following the shorter term exposures. However, upon retrieving the 2-week rangefinding inhalation study, which was not available to the Hazard ID Committee at the November 6 meeting, it became clear that cholinesterase inhibition and, particularly, nasal and laryngeal hyperplasia were evident after only two weeks, and thus the argument for not applying the uncertainty factor for short-term exposures could no longer be supported. (See Refs. O and CC) Indeed, Dr. Dourson expresses the view that the end points in question may not be particularly cumulative based upon similarities of responses in the 2-week and 90-day studies. I generally agree with Dr. Decker that range-finding studies perhaps do no often provide reliable information, but in this case the range-finding study is of higher quality than most such studies, and I believe to be suitable to the extent of revealing early onset of the nasal tissue effects, and cholinesterase inhibition. So while the reviewers did not clearly address the question as to whether the uncertainty factor should be used in the case of the short term (1-7 days) exposures, the question stands, begging a response from the Hazard ID Committee.

Question 3) **Should another study be required to identify the NOEL for the end points in question?**

Dr. Dourson: Yes (qualified). Suggests first using bench dose approach.

Dr. Hartung: "Not with rats on these issues." (p. 9)

Dr. Decker: Yes. "Common sense dictates that NOELs be identified." (p. 6)

Comments: Dr. Dourson evidently recognizes the need to more fully characterize the responses, i.e. a deficiency exists as it currently stands. Perhaps someone expert in this area could be commissioned to perform the tasks he suggests, and lets see what it shows. Dr. Hartung questions the utility of the inhalation study. However, the Agency requires the study and it is necessary that we assess the results. Dr. Decker most clearly enunciates what should be the Agency's position, which is to identify the NOELs on this very important end point for a very important route of exposure. It should be noted that in DER # 1 an extensive discussion is presented, indicating the very remarkable metabolic capability of the nasal olfactory epithelium and includes

discussion as to why malathion may be a good candidate chemical to elicit nasal effects following metabolic conversion by the nasal tissues.

Question 4) **Given the findings of nasal and laryngal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalational route (e.g., inhalation exposure for first 90 days of a two year study)?**

Dr. Dourson: Yes (qualified). As in his response to the previous question, he says first ask for mechanistic studies to understand nasal injury. Use extrapolation via cancer guidelines.

Dr. Hartung: No answer. Still questions utility of inhalation studies.

Dr. Decker: Yes.

Comments: Dr. Dourson recognizes the need to address the issue, but proposes as a first alternative pursuit of mechanistic studies and extrapolation techniques. Perhaps someone expert in this ared should be assigned the task and lets see what it shows, but I am not certain the most critical mechanism is identifiable with any certainty. Actual testing may be the best and perhaps only way to obtain satisfactory results. Dr. Decker is clear in his response that the study should be pursued. At other places in his response, Dr. Decker says: "The appearance of rarely-found malignant tumors in the nasal turbinates of 2 female rats should be a pointer that more animals should be tested to determine the incidence of said tumors in all dosage groups." (p. 2) We should note one of the rats in question had a carcinoma while the other had an adenoma of the olfactory epithelium. Were his suggestion to be followed, the inhalational route of exposure may be preferred, particularly if the study could be conducted in a manner acceptable to Dr. Hartung.

Question 5) **Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10X safety factor imposed under FQPA for the protection of infants and children?**

Dr. Dourson: No. Acknowledges study does not evaluate young individuals. Asserts the FQPA 10X factor to be a risk management tool.

Dr. Hartung: No.

Dr. Decker: No.

Comments: The external reviewers agree the study does not provide any support for discounting use of the 10X safety factor imposed under FQPA.

v Acute Neurotoxicity Study (Retinal Rosettes)

Supporting documentation: DER #s 9 and 10; References: L, M and P (pp. 1-2)

Question 1) **Should retinal histopathology data be submitted for rats in the intermediate dose group?**

Dr. Dourson: Suggests first requesting submission of slides in question and then decide whether to evaluate lower dose groups.

Dr. Hartung: Yes

Dr. Decker: Yes

Question 2) **Should histopathology slides be submitted for independent examination by the Agency's pathologist (for anatomic features comparison between control and treatment group lesion) as called for in the Data Evaluation Record (DER) for this study (a relatively simple request)?**

Dr. Dourson: Yes

Dr. Hartung: Yes (evaluate the matter by either approach)

Dr. Decker: Yes

Comments: All three reviewers share an opinion that additional work is indicated, the question is whether the work called for in both questions should be pursued. Dr. Decker says yes to both, while Dr. Dourson suggest that examining lower dose groups would be contingent upon the results of the independent histopathology examination proposed. Dr. Hartung advocates additional work to resolve the question. If it cannot be determined by the Agency's pathologist(s) whether the retinal finding in the high dose male group is dosing related, then it is important to acknowledge that the Guidelines require examination of lower dose groups.

VI Subchronic Neurotoxicity Study

Supporting documentation: DER #s 10, 11; References: D, P (pp. 3-4), Q, S, T, U and BB (pp. 12-14; 16-17; 20-22)

Question 1) **Given the contrast between the NOEL of 1575 mg/kg/day (HDT) for female rats on neurotoxicity end points in this FIFRA Guideline study and that of the LOEL of 38 mg/kg/day (LDT) in the published work on a different set of neurotoxicity parameters, does the published work provide adequate reason or evidence to require a developmental neurotoxicity Guideline study or another neurotoxicity study that embraces learning/memory, EEG, EMG , and possibly other neurotoxicity parameters not covered in the subchronic neurotoxicity Guideline study?**

Dr. Dourson: No. His reason resides in an opinion that if the study were performed, it would not likely yield a result that would infringe the RfD.

Dr. Hartung: Yes (implied), but questions the acceptability of Russian neurophysiology (EEG, EMG) assessments.

Dr. Decker: Yes

Comments: Dr. Dourson says no to this question for the reason that the LOEL of 38 mg/kg/day is not inconsistent with the cholinesterase NOEL in the 2-year rat study (a noteworthy observation in itself, attesting to the credibility of the non-Guideline study). He proposes applying a safety factor to the LOEL, which raises a concern analogous to that in the case of the inhalation study (Question IV), as to whether that is a suitable approach for these end points. The problems I find with this are: 1) the identification of an end point to be used for regulatory purposes, in this case the RfD based on cholinesterase inhibition, should be selected in

light of what the collection of Guideline studies reveal, i.e. all Guideline testing requirements should be satisfied, ideally each having been pursued to the point of rational conclusion. Each type of study in the Guidelines has its purpose; 2) Behavioral effects are of the highest order of importance; 3) If indeed the findings in Desi et al should be corroborated to show that behavioral effects, effects on neurophysiological parameters (e.g. EEG, EMG) and cholinesterase inhibition occur in neurotoxicity studies at doses comparable to those of cholinesterase inhibition in the Guideline 2-year rat study, the RfD derived from the latter would then have enhanced meaning among those persons who argue that cholinesterease inhibition itself, in the absence of other effects, is of questionable concern; 4) The Desi et al study did not identify NOELs on the very important parameters mentioned, and more than speculation should be employed to say at what doses effects terminate; 5) Desi et al was conducted in the female rat, and a question remains whether the Guideline 2-year rat study identified a NOEL for erythrocyte cholinesterase inhibition in the female rat.

Dr. Hartung says, prior to answering this specific question: "The assessment needs to incorporate the entire harmonized data set from all studies. It should not depend upon a search for single values, which are then treated without context." (p. 3) He also says: "It would be desirable to have at least a brief discussion of the interrelations of the various cholinesterases at different sites, their functions, and their diagnostic utility in relation to OP poisoning." (p. 4) This is a tall order as we all know, and this is why the implications of studies such as Desi et al indicating correlations between cholinesterase inhibition and other effects at low doses should not be dismissed out of hand. I am puzzled by certain elements of his response to the question at hand. He says: "The studies in DER #10 and DER #11 show no behavioral effects at dose levels significantly above dose levels associated with plasma cholinesterase inhibition, but they do show abnormalities in EEG and EMG recordings after 90 days of exposure." (p. 10) Actually, in Desi et al (DER # 11) effects on the behavioral parameters were observed at both doses tested (38 and 75 mg/kg/day) as assessed at 21 days, at which time statistically significant cholinesterase inhibition (approximately 20%) of the cerebral cortex was observed at both doses as well as statistically significant erythrocyte cholinesterase inhibition (also approximately 20%) at the 75 mg/kg/day dose level. Dr. Hartung says: "The spread between simple behavioral responses and cholinesterase inhibition argues against a need for further study." (p. 10) The converse of this is that further testing <u>would</u> be indicated if the said spread were small, or non existant, as is true in this case. He indicates his uncertainty as to what end points could be evaluated in the developmental neurotoxicity study, and would thus want assurances as to its interpretability before proceeding. This suggests, but does not say, he would support such testing were the test(s) meaningful.

Dr. Hartung questions the reliability of Russian neurophysiology, but without some reference to that literature with which to compare the work of Desi et al, it is difficult to appreciate any argument that the findings in Desi et al should not serve <u>at least</u> as a signal for definitive testing. It is documented in reliable sources that EEG is responsive to cholinergic agents, see Ref. U, and thus if EEG changes are noted in studies at doses close to, or particularly below, those that inhibit brain cholinesterase as assayed, this would be an important end point of probable regulatory concern.

Dr. Decker is firm in his recommendation that: "..... additional neurotoxicity testing to assess for effects on learning, behavior, and EEG and EMG evaluations."(p. 3), by the best methods available. He also says, with regard to DER #11: "I agree with the <u>Footnote</u> on page 13 that the neurotoxicity and neurobehavioral testing should be greatly expanded in scope, in light of developments in these areas during the past decade. The DER should be put 'on hold' until these changes are made." (p. 3)

In my view, the responses of Drs. Hartung and Decker support a requirement for additional neurotoxicity testing that would be designed to reconcile the contrasting findings between the published and Guideline subchronic neurotoxicity studies in question. It is important to mention here as discussed elsewhere in this

document that the publication by Kurtz (1977) (Ref. D) reveals a behavioral response to malathion within (actually below) the dose range that inhibited cholinesterase. The Guideline developmental neurotoxicity study, with some add-on testing, might be suitable to address the issue. While Dr. Dourson responds in the negative, his rationale does not incorporate or indicate consideration of the important issues being raised pertaining to neurotoxicity testing.

Question 2) **If the neurotoxicity findings in the published study are considered inadequate to trigger the additional Guideline testing, what criteria from published work, short of those upon which regulations could be directly based, might serve in that capacity? (Note: Moeller and Rider (1962), a journal publication with attendant Guideline deficiencies, has served for decades as the basis for a regulatable end point (RfD) for malathion, while the publication in question here is only being put forth as sufficiently definitive to require a study in the FIFRA Guidelines heretofore not perfomed.)**

Dr. Dourson: Defers to EPA's experts.

Dr. Hartung: No answer.

Dr. Decker: Suggests having a neurotoxicologist provide criteria.

Comments: The consesus opinion is to defer the question to neurotoxicologists. These also must be external peer reviewers

VII Cholinesterase Inhibition - Enhanced Sensitivity of Females

Supporting documentation: DER #s 1 - 3, 9, 10, 12 and 13; References: W, X, Y, Z and CC

Question 1) **Does the malathion data base support a conclusion that females are the more sensitive gender with respect to cholinesterase inhibition by this organophosphate?**

Dr. Dourson: Says maybe yes, but not so in the 2-year study now recommended by the Hazard ID Committee as the basis for the RfD.

Dr. Hartung: Says data are not presented in proper manner for his assessment.

Dr. Decker: Yes, more data needed to characterize the gender specific disparity

Comments: Dr. Dourson indicates while females may be more sensitive, they were not more sensitive than males in the 2-year rat study. It remains uncertain at this time as to just what the NOEL for erythrocyte cholinesterase inhibition may be in that study among females during the first 3 months of testing. Females were less sensitive on plasma cholinesterase inhibition in this particular study. It is unfortunate the data were not suitably displayed in order to gain the benefit of Dr. Hartung's opinion. Perhaps the possibility of follow-up with Dr. Hartung would remain in the event resoultion is not achieved without his comments. Dr. Decker considers the answer to be in the affirmative. In consideration of the responses to this question, and in view of the comments to the other questions in this section, a consesus exists that females are more sensitive.

Question 2) **What approach might be taken to estimate, from the data currently available, a correction factor to be applied to the NOEL derived from the Moeller and Rider study in male human subjects to**

afford equivalent protection for women?

Dr. Dourson: Equivocal. Does not support the effort if the human study is not used.

Dr. Hartung: Supports evaluating the data base for the male/female ratio of sensitivity.

Dr. Decker: Says not his area of expertise.

Comments: The reviewers appear to recognize the importance of the task, but are not certain how to approach it.

Question 3) **Should additional testing in animal models be required to further quantitate the gender specific disparity?**

Dr. Dourson: No, to the extent the human study is not used.

Dr. Hartung: Yes

Dr. Decker: Yes

Comments: A consesus exists to pursue the task. If the human study is retained as the basis for the RfD, it appears the consesus would be elevated to one of unanimity.

VIII Cholinesterase Inhibition - Chronic Dog Study

Supporting documentation: DER #s 1 and 4; References: B (p. 4), H, I and AA

Question **Knowing that the chronic dog study has no NOEL for cholinesterase inhibition and was considered unacceptable, should additional work, e.g. subchronic feeding study, be required to characterize cholinesterase inhibition in the dog?**

Dourson: No. However, his response appears to be predicated on use of an additional 3-fold uncertainty factor with the cholinesterase NOEL in the 2-year rat study.

Hartung: No.

Decker: Yes.

Comments: Dr. Dourson's response quite possibly would be different if the additional safety factor he recommends were not employed, particularly since he says elsewhere in his response: "I am not satisfied that the potential risk to humans is addressed with the data available in this review package." (p. 4)

None of the reviewers offer any comments in response to issues raised in Ref. AA, certain of which are summarized as follows: 1) The subchronic feeding study was waived in the 1988 Malathion Registration Standard contingent upon the performance of a chronic dog study. In waiving the subchronic study, there is an enhanced burden for completion of an acceptable chronic study; 2) There are species-related biochemical

similarities (absence of plasma carboxylesterase) to anticipate that the dog would respond similarly to man; 3) Cholinesterase methodology may be problematical in this 1987 study, and should be examined for conformity with the most current Agency standards; 4) The contrast between doses inhibiting cholinesterase in man and rat serves to indicate more definitive testing in a third species as FIFRA Guidelines intend; 5) The subchronic feeding study could possibly address the question of whether the manner of dosing is critical in the dog. The Hazard ID Committee should respond to these concerns

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B) December 17, 1997 Comments of B. Dementi on the December 17, 1997 Malathion Hazard Identification Assessment Review Committee Report.

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E) Pope, C. N. and Chakraborti, T. K. (1992) Dose-related Inhibition of Brain and Plasma Cholinesterase in Neonatal and Adult Rats Following Sublethal Organophosphate Exposures. Toxicol. 73, 35-43.

F) February 10, 1998 Letter of B. Dementi/Toxicologist to C. Swentzel/Chairman Hazard ID Committee.

G) A Two-Generation (Two Litters) Reproduction Study with AC 6,601 to Rats, June 28, 1990. MRID 41583401 (Selected Pages).

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I) November 10, 1997 Letter of B. Dementi/Toxicologist to C. Swentzel/Chairman Hazard ID Committee.

J) Two-Week (Range-Finding) Study of Aerosolized Malathion Administered byWhole-body Inhalation Exposure to the Albino Rat (July 20, 1993) (Selected Pages).

K) Organophosphate Action Update (January 1998) (Selected Pages). See "EPA Policies on Key Pesticide Policy Issues Clarified" (p. 2)

L) January 15, 1998 Letter of B. Dementi/Toxicologist to C. Swentzel/Chairman Hazard ID Committee.

M) March 28, 1995 Letter of B. Dementi, "Malathion Acute Neurotoxicity Study; MRID# 431467-01"

N) Toxicology Endpoint Selection Process - A Guidance Document Prepared by Jess Rowland, Health Effects Division, Office of Pesticide Programs. February 1997.

O) March 10, 1998 Letter of B. Dementi to Jess Rowland.

P) November 20, 1997 Letter of B. Dementi to C. Swentzel/Chairman Hazard ID Committee

Q) Desi, I. et al (1976) Toxicity of Malathion to Mammals, Aquatic Organisms and Tissue Culture Cells. Arch. Environ. Contam. Toxicol., 3, 410-425.

R) November 25, 1997 Letter of B. Dementi to C. Swentzel/Chairman Hazard ID Committee

S) May 4, 1995 Letter of R. MacPhail to J. Doherty and B. Dementi, Health Effects Division.

T) May 11, 1995 Letter of Brian D to Karen

U) Goodman and Gilman's The Pharmacological Basis of Therapeutics (1980) Selected Pages.

V) A Range-Finding Teratology Study with AC 6,601 in Rabbits (MRID 470088-012), Selected Pages Including Tables 3: Summary of Doe Body Weight During Gestation and Table 4: Summary of Doe Body Weight Gain During Gestation.

W) April 29, 1997 Letter of B. Dementi to E. Budd.

X) August 10, 1992 Letter of D. Allemang to L. Schnaubelt; October 8, 1992 Memorandum of B. Dementi to J. Edwards.

Y) January 22, 1993 Memorandum of B. Dementi to J. Edwards.

Z) Malaoxon Chronic Toxicity/Carcinogenicity Study: Selected Pages From DER #2.

AA) March 20, 1998 Letter of B. Dementi to C. Swentzel.

BB) Dementi, B. (1997) Cholinesterase Literature Review and Comment. Part A

CC) March 16, 1998 Letter of B. Dementi to J. Rowland.

MEMORANDUM April 8, 1998

To: Henry Spencer, Ph.D.
Manager, External Peer Review
Science Analysis Branch
Health Effects Division

From: Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch I

Attached you will find questions (I-VIII), plus supporting reference documents, I am submitting to accompany the Hazard ID Committee report that will be going out for external peer review.

ATTACHMENT 13: Letter from B. Dementi - July 29, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

July 29, 1998

Re: Malathion External Peer Reviews, Follow-up Questions

This is an addendum to my memorandum to you of July 27, 1998.

Please find appended copies of letters from Dr. Michael Dourson (July 17, 1998) and Dr. Walter Decker (July 21, 1998) in which these external reviewers respond to additional questions posed by me after receiving their initial evaluations.

In Dr. Dourson's response to my first question, he expresses concern regarding the reliability of reported decreased pup weights during days 7 and 14 of lactation, which he says was due to chance, but concludes the LOAEL/NOAEL = 5000/1700 ppm for pup body weight changes based on findings at day 21 of lactation. This is in agreement with pup LOEL/NOEL identified in the DER. It should be noted that the pup weight decrements in question at days 7, 14 and 21 were all statistically significant findings. However, Dr. Dourson did not address clearly my real question, which is whether the evidence of greater sensitivity of pups versus adults in this study can be reliably discounted using the arguments put forward by the Hazard ID Committee without a showing of malathion in the milk (and how much is there) and without any data to indicate how much solid food pups may have actually consumed during lactation.

In Dr. Dourson's response to my second question, he asks whether the effect on avoidance behavior was statistically significant. Kurtz (1977) says that it was statistically significant, $p < 0.02$. (p. 590) Further, the publication says: "... significant behavioral decrements were found at dosages producing only negligible changes in ChE activity: The median avoidance latency of the group tested 1 hour after injection with 50 mg/kg was 12.2 sec compared to 0.6 sec in the control group, but ChE activity of this group was greater than 90% of control values for all three ChE measures." (p. 591) Consideration of this and other information in the Hazard ID Committee reference materials, and Dr. Dourson's comments in his item 3 of question 2, would indicate some recognition on his part of the need for conducting the developmental neurotoxicity study on malathion. He says at the very least, he would ask that the i.p. study be repeated with more animals and more behavioral tests. Clearly the concern is real.

In Dr. Decker's response to the question posed by me regarding the inhalation study, he says no to the 1/3 LOEL, and advocates an interim 1/10 LOEL for the inhalation study, while assuming, " of course, that further testing will be forthcoming to determine a NOEL, ..."

Brian Dementi
Toxicologist
Toxicology Branch I/HED

ATTACHMENT 14: Letter from B. Dementi - August 3, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

August 3, 1998

In preparation for the August 18, 1998 Hazard ID Committee meeting on malathion, I have a few additional comments regarding the interpretation of the malathion two-generation reproduction study in the Sprague-Dawley rat (MRID 41583401) (DER #5)

The December 17, 1997 Hazard ID Committee report covering the November 6, 1997 meeting to consider malathion says: "For parental systemic toxicity, the NOEL was 5000 ppm (394/451 mg/kg/day in M/F) and the LOEL was 7500 ppm (612/703 mg/kg/day in M/F) based on decreased P generation body weights during gestation and lactation and decreased F1 pre-mating body weight." (p. 15) The problems I have with this, as gleaned from the DER of the study, are explained as follows: 1) Parental (dam) body weight was not affected at any dose level during either of the two F1 lactation periods, i.e. for litters F2A and F2B, as recorded in Table 3 (p. 11) of DER #5, copy appended; 2) Parental (dam) body weights were significantly less in the 7500 ppm dose group for both of the F0 lactation periods, i.e. for litters F1A and F1B as recorded in the same Table 3. However, in the case of both of these F0 lactation periods the effects were most remarkable on lactation day 0, a day which follows immediately on the heels of delivery and more properly should be viewed as a manifestation of effects incurred during gestation and delivery. The meaningful period for assessing dam body weight effects of/during *lactation* rests with what happens *after* Day 0, i.e. on days 7, 14 and 21 in this case. As I examine this data during both of the F0 lactation periods, I observe considerable recovery of dam body weights by day 7, and that *changes* in body weight assessed across the 21 day period (e.g. days 7-14, 7-21, 14-21) in all dose groups appear to be essentially unaffected at any dose level (I say this without the benefit of statistical analysis, which I recommend be done). To the extent that the body weights in the 7500 ppm dose groups remain less than the control post day 0 is arguably a carry over of the Day 0 deficit, since there is little or no evidence at any subsequent time point of further erosion of body weight. Again, during both F0 lactation periods, dams show evidence of recovery post Day 0. My view is that dams in the 7500 ppm dose group were affected during pregnancy insofar as indicated by body weight deficits on lactation Day 0, but no conclusive evidence exists to show *during* lactation that dams were affected at any dose level in any of the four lactation periods under study; 3) Decreases in dam body weight during *gestation* in my view cannot be interpreted to be uniquely parental/dam effects; 4) During the pre-mating period there were no effects on F0 male or female body weights, Table 1 (p. 9) of DER #5, copy appended. However, there were statistically significant body weight decreases in both F1 males and females at the 7500 ppm dose during the pre-mating period. While this may suggest a parental effect at 7500 ppm, it must be recognized that F1 animals, unlike F0 animals, were exposed to malathion in utero and, hence, effects cannot be divorced from a possible fetal/developmental etiology. So to the extent that the reproduction study is employed to differentiate possible differences in sensitivity between young/developing individuals and adults, as required under FQPA, effects on F1 parental animals are to be of questionable usefulness. In the case at hand, the fact that body weight effects were observed in the F1 animals at 7500 ppm during premating, but not in F0 males or females during premating is supportive of a possible adverse effect of the test material on F1 animals during development, manifested as an enhanced adult sensitivity.

The bottom line is that there are no unencumbered body weight data in this study that shows an adverse effect of malathion at any dose level on adult animals apart from possible effects on the developing animal. Evidence which has been cited in support of an effect at 7500 ppm is indefensible. Effects on body weight

during pre-mating were only on F1 animals, which were exposed during development. During lactation, there were either no effects (F1 lactation periods), or effects seen at Day 0 (F0 lactation periods) tended to recover and/or got no worse *during* lactation and, hence, cannot be said to represent effects peculiarly on dams divorced from possible consequences of effects on the developing individuals. Similarly, dam body weight changes during gestation cannot be used to deminstrate a peculiar effect on adult animals.

So, as I have said previously in my letters to Jess Rowland (December 17, 1997; Ref. B) and to you (February 10, 1998; Ref. F), body weight changes and other parameters evaluated in reproduction and developmental toxicity studies do not provide adequate information to identify possible greater sensitivity of young/developing animals versus adults. But even to the extent that body weight changes in adults and offspring evident in the two-generation reproduction study on malathion have been used for this purpose, closer examination of the DER does not reveal any indisputable or *reliable* evidence of an effect of malathion on body weight changes in adults at any dose level, either during *gestation*, *lactation* or *pre-mating* periods as claimed in the Hazard ID report. Effects on offspring occurred at 5000 and 7500 ppm, and possibly at all doses of the F1A litter during lactation in terms of body weight deficits. The study thus supports a greater sensitivity of the developing organism.

Our experts on reproduction toxicology should be invited to examine the study closely and comment on the views I have expressed for the benefit of the Hazard ID Committee.

Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch I/HED

ATTACHMENT 15: Letter from B. Dementi - August 10, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

August 10, 1998

As explained in my letter to you of August 3, 1998, closer examination of the DER (#5) for the 2-Generation Reproduction Study for malathion does not reveal any indisputable or *reliable* evidence of an adverse effect of malathion on body weight changes in parental animals at any dose level, either during *gestation*, *lactation* or *pre-mating* periods as claimed in the December 17, 1997 Hazard ID report. This would mean that for the said study the parental toxicity NOEL $\geq$ 7500 ppm (HDT), while the developmental NOEL/LOEL = 1700/5000 ppm.

Again, in preparing for the August 18, 1998 meeting, I have now examined the Study Report of the 2-Generation Reproduction Study for further details and must advise the Committee that the **Study Report** (MRID 41583401), in contrast to the DER (#5), concluded there was no adverse effect on parental animals: "Thus, in this two generation reproduction study in rats involving continuous treatment with AC 6,601 in the diet, the parental no-observed -advers-effect level (NOAEL) was 7500 ppm and the NOAEL for developmental toxicity was 1700 ppm." (p. 6) The principal reason for this discrepancy between the DER and the Study Report rests with the reporting of parental body weight data. The DER reported only mean body weights of parental animals at critical time points, such as pre-mating, while the Study Report provided data showing changes in body weight as well. Thus while body weight in a 7500 ppm parental group may have been less than the control, body weight changes during the period in question were unaltered.

For example, in the case of pre-mating parental body weight data, for the F0 parental animals there were no treatment related effects of dosing on body weight. However in the F1 parental animals, while mean body weight in the 7500 ppm group was less than that of controls, there was no effect during this period on body weight *gain*, a finding neither discussed nor noted in the DER (#5). The Study Report says the following with respect to the F1 parental animals: "In Group V (7500 ppm), mean weekly weight data for males and females during the pre-mating treatment period were lower than control and these differences, throughout this interval, were statistically significant. Mean weight gains, however, over the entire 10 week pre-mating period for these Group V animals (both sexes) were comparable to control data. Thus, while Group V animals initiated the pre-mating treatment period smaller than control animals, and ended the period smaller, the weight gain experienced by these two groups over the entire period was considered comparable. Thus, no adverse effect of treatment up to a dietary level of 7500 ppm was evident from weight gain data during the pre-mating treatment periods for either parental generations (P1, F1)." (p. 28 of the Study Report). In my view this assessment in the study report is supported by the data presented in that report, is entirely correct and indicates a need for revisions to the DER (#5) to present a more satisfactory interpretation of the findings where the relative sensitivities of adult versus young/developing animals is concerned. This need is more critical now that the reproduction study is being relied upon to make such destinctions as required under FQPA.

Similarly, the study report provides data showing that mean weekly body weights during the mating and post-mating periods for F0 male animals to produce the F1a and F1b litters, were comparable between the control and treated groups. By contrast, in the case of F1 males (which unlike F0 animals were exposed in utero),

weekly body weights during the mating and post-mating periods to produce the F2a and F2b litters were statistically significantly lower in the 7500 ppm group than the control, but are only consistent with the lower weights seen in this group during the pre-mating period. The study report says : "Thus, no adverse effect of treatment up to a dietary level of 7500 ppm was indicated from weight gain data for males during the mating and post-mating intervals for either the P1 or F1 generations." (p. 33 of the study report) Again, I find this conclusion entirely supportable by the data in the Study Report, which is simply not conveyed forward in the DER (#5).

The bottom line to all this is that the data in the Study Report do not support a conclusion that parental animals were affected at any dietary level of malathion tested as gleaned from body weight data. The DER (#5) should be revised to reflect these findings and is a matter that should be commented on by the Committee.

This further supports what I indicated earlier, namely, to justify removal of the FQPA imposed 10X factor, there is a larger gap between the developmental NOEL/LOEL (1700/5000 ppm) and the parental NOEL ($\geq$ 7500 ppm) to be explained away by the Hazard ID Committee than was considered to be the case at the November 6, 1997 meeting.

Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch I/HED

ATTACHMENT 16: Letter from B. Dementi - August 17, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

August 17, 1998

In response to your memorandum of August 14 concerning the format for the August 18 meeting of the HIARC (copy appended), I am concerned that the discussion may be restricted to the eight topics generated by me that were submitted for external review. I say this because Dr. Hank Spencer, the external peer review coordinator, introduced certain preliminary questions that were also responded to by the three scientists in question, Drs. Michael Dourson, Rolf Hartung and Walter Decker, that require assessment by the Committee. These questions pertained to the acceptability of the various malathion DERs, whether critical effects were chosen in the various studies and whether the data base is complete. One of my principal concerns as expressed in my December 17, 1997 comments to Jess Rowland, Ref B in the background package, was that of whether there are data gaps in the malathion data base. As the Committee is aware, removal of the 10X safety factor under FQPA for the protection of infants and children requires a complete data base. In consideration of the fact that the external reviewers had much to say regarding the adequacy of the data base and data gaps, I had planned to mention this to the Committee.

Pertaining to the acceptability of the malathion data base, the following are noteworthy statement rendered by the external reviewers.

Dr. Dourson says "The lack of the monitoring of the critical effect in the developing offspring, and specifically, the lack of such measurment of RBC cholinesterase inhibition in the 2-generation study is a data gap....." (p. 3) "The specific question to be addressed with these data are whether or not the NOEL of the likely critical effect after 1 day exposure is determinable. The available data in this review, including the developmental studies in rabbits, do not allow this question to be answered." (p. 3) "No, the data on which to make this determination are absent." (p. 5) "However, I believe that the rat NOEL should be further divided by a 3-fold uncertainty factor to account for deficiencies in the data base...." (p. 8) "However, it does not test females, so the NOEL/LOEL range could be lower." (p. 8) His responses to both questions IV and V calling for additional information indicate his recognition of the existence of additional data gaps. A most significant statement made by Dr. Dourson reads as follows: *"I am not satisfied that the potential risk to humans is addressed with the data available in this review package." (p. 3)*

Dr. Hartung, beyond saying that a toxicology data base is never complete (p. 4), does not particularly address the question specifically for malathion. He does say the following: "The available data is inconclusive whether a single dose, administered during a day of maximum sensitivity would be able to elicit the observed response, or whether cumulative dosing is required." (p. 5) "This requires an analysis of the detailed cholinesterase methodology." (p. 7)

Dr. Decker: "The appearance of rarely-found malignant tumors in the nasal turbinates of 2 female rats should be a pointer that more animals should be tested to determine the incidence of said tumors in all dosage groups. The tumors should be further histologically defined." (p. 2) Along these same lines, he indicates that these findings "...demand further testing in a larger group of animals in all dosage groups." (p. 4) "The finding that the increased numbers of hepatocellular tumors observed in the male mice at 100 ppm as compared to the

lower numbers of such tumors observed at 800 ppm is not interpretable, in my opinion. Rather, this part of the study should be repeated. The rest of the study seems to follow the Guidelines well, and appears to be scientifically valid." (p. 2) "I agree with the EXECUTIVE SUMMARY that this study is not acceptable and does not satisfy Guideline 83-1 for a chronic toxicity study in dogs because NOELs were not established for cholinesterase activity inhibition for plasma and erythrocytes in either sex." (p. 2) "Lacking an answer to this question, I would recommend that this DER be changed from CORE MINIMUM to UNACCEPTABLE for the section of the report on eye histopathology." (p. 3) "Although this study appears to satisfy the requirement of Guideline 82-7 for subchronic toxicity determinations, it was correctly pointed out in the Study Classification section that other published data indicate possible evidence of neurotoxicity on parameters not assessed in the 82-7 Guidelines. I recommend a thorough literature search on theses and that the results be used to construct additional specific neurotoxicity testing to assess for effects on learning, behavior, and EEG and EMG evaluations." (p. 3) "I agree with the Footnote on page 13 that the neurotoxicity and neurobehavioral testing g should be greatly expanded in scope, in light of development in these areas during the past decade. The DER should be put "on hold" until these changes are made." (p. 3) "This study seems to be generally acceptable, but does not satisfy all requirements of Guideline 82-4, since no NOEL was established for plasma and RBC cholinesterase inhibition in female animals or for microscopic lesions of the nasal cavity of the larynx in both sexes." (p. 3) "I recommend that Dr. Dementi's suggestions be actively pursued, that is more studies are needed to fill in data gaps." (p. 4)

These various views rendered by the external scientists serve to underscore my expressed opinion that it cannot be claimed, as was done in the December 17, 1997 report of the HIARC that "The toxicology data base is complete and there are no data gaps." (p. 18) This latter statement is offered as satisfying one of the requirements under FQPA that must be met before the 10X safety factor, imposed for the protection of infants and children, can be removed. I doubt very seriously that Congress intended that anything other than fully acceptable studies, with no data gaps of the nature identified by the external reviewers, could be used to satisfy this very important criterion for removing the said 10X factor. I must express additional concerns I have regarding the procedures to be followed for the August 18 meeting as expressed in your August 14 memorandum. As you know I have many contrary views respecting those of the HIARC over the adequacy of the malathion data base, and I must insist upon the freedom to express my views. There is much food for discussion resident both in the several memoranda I have addressed to the Committee since the November 6 meeting, and in the comments of the external reviewers. I consider it unfortunate that so many issues are contemplated for this one meeting, and am concerned therefore that each issue may not be accorded the time needed in the press to cover all the issues in one meeting. I am also concerned over the assignment of certain members of the HIARC to the various questions, as this may have a negative effect on the extent to which other members of the Committee evaluate all of the issues, i.e. too much reliance of the Committee as a whole may be placed on the opinions rendered by the one principal reviewer in each case. Of course, I would hope and trust this would not be the case, but I would also hope that each member of the Committee would be invited to express his/her views on any and all questions after having thoroughly studied the full data base. As I have found it to be true at various HED Committee meetings, issues/questions often arise that have no adequate response at the time, and certainly in my case, I sometimes don't provide the quality of answer I might given more time to reflect on various issues, as these often are complex. Hence, there simply must be opportunity for follow-up after these meetings, before final reports go out. Nothing, including the press of time, should preclude gathering and expressing the facts, no matter how far a hearing has advanced, where public health matters are of concerned.

Brian Dementi, Ph.D., DABT
Toxicologist, Toxicology Branch I/HED

ATTACHMENT 17: Letter from B. Dementi - September 24, 1998

Clark Swentzel, Chairman
Hazard ID Committee
Health Effects Division

September 24, 1998

Please find appended a copy of the following journal publication from the open literature: Mendoza, C.E. (1976) Toxicity and Effects of Malathion on Esterases of Suckling Albino Rats. Toxiol. Appl. Pharmacol., 35, 229-238.

This is being offered for possible inclusion under section III (2) (iii) "Information from the open literature" (p. 16) in the December 17, 1997 "Report of the Hazard Identification Assessment Review Committee" on malathion, as relevant to the Determination of Sensitivity for FQPA Considerations

Perhaps the person who assimilated the literature review section of your report would find this publication both interesting and relevant, and wish to incorporate it in the review. I have read the article, which leads me to conclude that it provides information indicating younger animals to be more sensitive to malathion, but have not had time to review it.

Brian Dementi, Ph.D., DABT
Toxicologist
Toxicology Branch I/HED

ATTACHMENT 18: Letter from B. Dementi - November 5, 1998

Clark Swentzel, Chairman
Hazard Identification Assessment Review Committee
Health Effects Division

November 5, 1998

Comments I would offer to the October 27 draft report of the August 1998 Malathion Hazard Identification Assessment Review Committee are offered below.

I SPECIFIC COMMENTS

P. 1, paragraph 2: The August HIARC meeting occurred on August 18, 20 *and 27*, 1998.

P. 1, paragraphs 2 and 3: You say HIARC evaluated the comments and responses provided by the external peer review. Actually the statement should include affirmation of the fact the committee was also considering the comments presented in some fourteen memoranda on toxicology issues submitted by Dr. Dementi, many of which served as the impetus for the external peer review and in fact were addressed by the peer review members. You cannot divorce these memoranda from the deliberative process in presenting an historically correct record. You should also say at this point that these memoranda by Dr. Dementi constitute part of the record of the deliberative process.

P. 2: Karle Baetcke was not in attendance at any of the meetings I attended on August 18, 20 or 27.

P. 3, paragraph 2: You say "Following that meeting, the Agency conducted an external peer review of a number of issues related to hazard identification for malathion." From whence did those issues arise? For the benefit and enhancement of the understanding of your audience, this statement should be more forthcoming in laying down the historical record and rationale for that external review. Accordingly, your statement might be rephrased thusly: *"Following that meeting, the Agency pursued the external peer review mechanism to address the number of issues raised by HED's malathion toxicologist following the November 6, 1997 HIARC meeting."*

P. 3, paragraph 2, 3d line: *experts*

P. 3, paragraph 3: August 18, 20 *and 27;* responses; "..... of the external peer review panel *and Dr. Dementi.*"

P. 3, paragraph 4: *Michael; Rolf;* in addition to the eight major topics, you should acknowledge the preliminary questions concerning the general acceptability/completeness of the data base posed by Dr. Hank Spencer, HED's external peer review coordinator. You say the Panel received all pertinent reference materials. However, you should go a little further in informing your audience as to what these materials were, namely study DER's, one-liners and Dr. Dementi's memoranda and set of questions.

P. 4, paragraph 2: Delete *Do the rabbit*

P. 4, paragraph 4: *range-finding; main rabbit; considered*

P. 4, paragraph 4: Under HIARC's justification for the acute oral (one-day) end point, see my comments on

page 6 (paragraph 4) of this document analogizing to oral exposure HIARC's former assumption that no effects would have been expected early into inhalation exposure.

P. 4, paragraph 5: *the acute RfD; These decreases;*

P. 5, paragraph 3: *error in equation, 50 mg/kg/day/100 (UF) = **0.50** mg/kg/day. It is noteworthy that this dose is 21.7 (12.5)-fold that of the RfD of 0.023 (0.04) mg/kg/day, depending upon whether the human or rat study serves as basis for the RfD.*

P. 5, paragraph 4, last line: ....*study* sufficiently....

P. 5, paragraphs 5 and 6: Your report does not provide the Panel's response or HIARC's conclusion relevant to the all important question 3) c) namely, " Is the data available in the developmental study sufficiently reliable to discount the 10X safety factor required under FQPA?" Of course, the Panel's opinion was unanimous that it is not.

P. 6, paragraph 2, last line: ".....evidence (of parental toxicity) is not strong." More needs to be said here by way of qualifying this remarkable statement. If the evidence is "not (as underscored) strong", how can it satisfy as *reliable* data for the protection of infants and children as specifically required under FQPA? You must explain that obvious anomaly. In my view as expressed at the HIARC meeting, the study does not satisfy as showing a parental effect at any dose level which means there are pup body weight effects at two doses in the absence of parental toxicity, thus establishing greater sensitivity of the young and developing individual, the specific concern of FQPA. It was my understanding at the HIARC meeting on August 20, that Dr. Dapson was going to provide a written supplemental review of the data in this study after it was pointed out that the author of the MRID study report had concluded there were no parental effects at any dose level and that this was based on body weight *gain* data presented in the study report that had *not* been incorporated into the DER. Furthermore, the study author had concluded that offspring were adversely affected at both the top and penultimate dose levels. I must ask the question as to whether any re-review of this particular aspect of the study has been undertaken by the committee that drives your qualifying remark about the finding *not* being strong. Any rationale supporting this new claim must be presented for all to see.

P. 6, paragraph 5: Your statement in bold print is troubling to me. The statement as a whole does not bespeak of the kind of certainty that I believe was the intent of Congress in calling for *reliable* data. Furthermore, I do not recall this statement as consistent with the tenor of the discussion held on August 20, but rather strikes me as some sort of new rationalization developed since that meeting. Have there possibly been other meetings of the HIARC held since August 27? You also say in this paragraph that "The presence of the chemical in the milk is a generic assumption .....(unless we have data to show otherwise)...." Your record must show that at the August 18 meeting, when this issue was first visited, Dr. Protzel left the meeting to retrieve the residue chemistry metabolism study. That study, performed in the goat, revealed only two non-cholinesterase inhibiting metabolites of malathion, i.e. malathion was not present in the milk. Furthermore, I subsequently spoke with Mr. Bill Smith, malathion team chemist, who confirmed via the spoken word, that malathion is not a residue in milk. So the condition for your generic assumption to apply has not been met by the Agency's actual testing procedures as required under Residue Chemistry to set tolerances. This information should be recorded in your report, particularly the effort on Dr. Protzel's part as that actually took place at the meeting. What's more, the committee was reminded on August 20 while the milk issue was still under discussion that Dr. Protzel had obtained the data at the previous meeting on August 18 and it was negative for malathion. Furthermore, the committee needs to revise its conclusion as to the use of the milk arguement in discounting relative sensitivities of young and developing individuals versus adults in the reproduction study. I wish to reaffirm here the view I expressed at the August meeting that the reproduction study reveals a greater

sensitivity of the young and developing individual, and that arguements to the contrary simply "don't hold water", paricularly so in view of Congess' qualifier in FQPA regarding the need for *reliable* data.

P. 6, last paragraph: I don't affirm this line of reasoning as having taken place at the August meeting. However, if it was, the question needs to be referred back to the FQPA Safety Committee. I am certain that committee would want the correct data in place for the rendering of its opinions, and that committee should also now be privileged to have the benefit of the external reviewers' opinions in addressing the issues. However, contrary to this you do say on p. 1 that the HIARC committee "....addressed the sensitivity of infants and children from exposure to malathion as required by the Food Quality Protection Act (FQPA) of 1996." So to the extent HIARC did in fact perform this assessment as claimed, you should not disown this responsibility by placing it on the shoulders of the FQPA Safety Committee as you're doing on p. 6. **All things said, the Agency's obligation is to the protection of the public health, and the scientific facts are what count in setting end points and applying safety factors, regardless of which committee assumes the burden of rendering the judgement.**

P. 7, paragraph 3: The committee's conclusion does not address the question of FQPA's insistance upon *reliable* data. Does this study meet the test of providing *reliable* data for the protection of infants and children under FQPA. The expert panel has said no. How can the committee justify to the public a decision differing from this in discounting the 10x factor required by Congress?

P. 7, between paragraphs 4 and 5: "Panel's Response" to this very critical question seems to have fallen through the cracks. In response to this question, you Panel's Response should record that Dr. Dourson suggested a 3X safety factor as opposed to 10X, while acknowledging 10X may still be useful as a management tool. Drs. Hartung and Decker say no, though Dr. Hartung insists offspring must be shown to be less sensitive. Also the external reviewers were not aware of the more recent concern that the DER for this study did not address the study author's observations that body weight *gain* data, not shown in the DER, do not support a conclusion that adult animals were affected at the highest dose. Nor were the external reviewers aware that malathion has been shown not to be present in milk, thus removing one principal reason HIARC employed to discount differences in sensitivity between offspring and adult animals in the said reproduction study.

P. 7, paragraph 5: Among the basic Guideline studies, only the developmental toxicity and reproduction studies assess relative sensitivities of young and adult animals. You need to make that clear here. To the extent the reproduction study fulfills this role, the external reviewers have said the study does not provide such reliable data. That taken in concert with data showing greater sensitivity of young animals in this study, as I believe it does, leads me to doubt very seriously the public would take much comfort in the generic issue arguement being waged here to discount the absence of satisfactory data to make the needed destinctions between young and adult animals.

P. 8, paragraph 5, line 5: In order to adequately convey to your audience the assessment of Dr. Dourson when using his quote, firstly the quote should read as follows: "..... *principally because the critical effect was not monitored in the two-generation reproduction study in a potentially sensitive subgroup (i.e. young rats).*" Secondly, Dr. Dourson is speaking here of but *one* critical effect, namely cholinesterase inhibition. My claim in identifying that particular effect as cholinesterase inhibition is supported by the following statement of Dr. Dourson: "The lack of the monitoring of the critical effect in the developing offspring, and specifically, the lack of such measurement of RBC cholinesterase inhibition in the 2 generation study is a data gap that can best be addressed through the use of a 3-fold uncertainty factor when determining the RfD." (page 2 of Dr. Dourson's June 3, 1998 submission). It is important that you make your audience aware of the identity of the effect (cholinesterase inhibition) because it is the basis of the RfD, and that Dr. Dourson considers it a data

gap. The full weight of his testimony should be conveyed here. **Also, since you have quoted from Dr. Dourson, a balanced approach would necessitate quoting the other external reviewers.** These quotes are not long. *Dr. Hartung*: **"No. The human is the correct species of concern. Substituting a rodent introduces many more uncertainties than those produced by minor deficits in the analysis of chemical purity or concern about statistical precision."** (p. 7 of his 6/3/98 comments); and **"Look at what you are doing! Here you are willing to accept a study for which you are also willing to mess around with another factor of 10X, just because the statistical data are neater. In the process you are willing to discount human data, even though it is extremely unlikely that the equivalent statistical uncertainties for the human will reach anywhere close to 10X."** (p. 8 of his 6/3/98 comments). Note he addresses the purity question, and I advised the committee that the human study in question, Moeller and Rider, while not stating the purity, did claim it to be American Cyanamid malathion, the purity of which was known in the industry at the time. Furthermore, at the committee meeting there was an extensive discussion of the fact that the rat may be a poor surrogate for man, based upon differences in carboxylesterase profile in rat versus man. The committee even concluded on August 18 to impose the additional 3-fold uncertainty factor, which the committee reversed on August 20 because the issue may relate to other pesticides where it has not been addressed. *This aspect of the deliberations finds no entry in these draft minutes. Dr. Decker*: **"Additional testing should be required in the male <u>and</u> female rat before any thought is given to replacing the human data relied on to establish a RfD."** (p. 5 of his 6/11/98 comments)

You should say here something such as: *In summary, two external reviewers were firm in recommending against switching to the rat study, while the third member favored the rat study, contingent upon imposition of an additional 3-fold uncertainty factor. The committee is ignorant as to the latter's views regarding the use of the rat versus the human study in the absence of an imposed additional uncertainty factor.*

P. 8, paragraph 6: For the full understanding of your audience, remembering the importance of *transparency* in our products, you should say something at this point to the effect that: *In this HIARC decision, the recommendations of **all** external reviewers were discounted.*

P. 9, paragraph 3: The Panel's Response as described is incorrect in light of the following: 1) *Dr. Dourson* advocated 10X as opposed to 3X. 2) *Dr. Decker*, in his follow-up response of 7/21/98 says: **"Based on my experience (43 years in the field of toxicology), Reference N (TES Process), and the letter from Dr. Dementi (July 9, 1998), I doubt that the 1/3 LOEL is adequate to account for the absence of a NOEL. At the present time it would seem prudent to use 1/10 LOEL." I assume, of course, that further testing will be forthcoming to determine a NOEL, at which time this safety factor should be reexamined."** 3) *Dr. Hartung* says: **"This fine-tuning is unwarranted because of major species differences in exposure scenarios."** This should be interpreted to mean that fine-tuning, 3X or 10X, in his view cannot address the inadequacies. It cannot be taken to mean he opposes increasing the factor from 3X to 10X. Indeed, given his expressed views, proper testing is indicated, but lacking that and until proper data is in place, the implications of his words *convey to me* that he would consider 10X as preferred for public health protection, although he does not actually say that. The bottom line is that two reviewers, a consensus, supports the imposition of a 10X safety factor, while the views of the third should be suitably qualified in your report and cannot be simply cited as "one member recommended against the use of an additional UF", left to be interpreted to mean Dr. Hartung sees no need to increase the uncertainty factor because the study is adequate as it stands. Again, *transparency* of your presentation is the issue here regarding Dr. Hartung's comments.

P. 9, paragraph 4: This HIARC conclusion is incompatible with my notes and recollections of events that transpired at the August 27 HIARC meeting, a meeting which, incidentally, is not even acknowledged in these minutes as having occurred. *This is peculiar and of great concern to me.* At that HIARC meeting, in my

witness the committee's designated "expert" recommended and the committee adopted raising the UF from 3 to 10. There is no mistake in this. Does this conclusion possibly reflect deliberations of the committee that took place at another time in my absence? If so, the minutes of any such meeting, including the date, who was present, etc. should also be a matter of record and noted here, for the sake of historical accuracy, if nothing else. If another meeting after August 27 did *not* occur, are these draft minutes to be viewed as perhaps anticipatory of what is yet to be presented, suggesting selective prior knowledge, in which case they are not all minutes of past events and should require no response at this time, for how can one be expected to comment on an event he never witnessed or attest to events yet to occur.

According to my witness, the HIARC Conclusion should say, for example, *The HIARC concluded that the Margin of Exposure should be increased from 3X to 10X, for both Intermediate and Long-Term inhalation exposures.*

P. 9, paragraph 5: The rationale presented here relates to question 2. Since in actuality, you did not address question 1 in your conclusion, the rationale for your decision as presented in this paragraph 5 is irrelevant and immaterial insofar as it puportedly relates to question 1.

P. 9, paragraph 7: *members; suggest.*

P. 9, paragraph 8: HIARC Conclusion should record that the committee decided to invoke an MOE for short term acute risk assessment for the reason that the effects of concern were seen in a two-week study. However, the conclusion should also reflect the August 27 decision to invoke the same 10X factor for short term as for the intermediate and long term endpoints.

As an aside I should note here the committee was too quick back in November, 1997 to deny the need for an MOE, by assuming, in the absence of short term data, that effects would not occur in the short term. I should also note that Dr. Dourson's comments suggesting the inhalation data do not support cumulative effects for cholinesterase inhibition, leading him to say "..... that an extra uncertainty factor for potential cumulative effects is not needed." (p. 10 of his 5/29/98 comments) are not only germane to inhalation exposure MOEs, but to the question of the committee's acceptance of an acute, one-day RfD as high as 0.50 mg/kg/day (based on non-cholinesterase data) as contrasted with the longer term cholinesterase data derived RfD of 0.023 (or 0.04) mg/kg/day, a 21.7 (12.5)-fold difference, wherein again it is being assumed, in the absence of short-term oral data, that cholinesterase would not be as responsive over the course of 1-7 days as it is beyond this time frame. Protection of the public health demands more than assumptions in setting these important end points, whether they be inhalational or oral end points. Until short-term (1-7 day) oral cholinesterase data are available, one RfD for all time points should be employed.

P. 10, paragraph 3: In my witness, the HIARC Conclusion offered here is inconsistent with my understanding at to the committee's conclusions rendered August 27. According to my records, the committee imposed an uncertainty factor of 10 on all three end points. Furthermore, the committee decided to require another inhalation (nose-only) study in the rat. The requirement for this study was driven primarily by the nasal tissue effects, for which there was no NOEL in either study. I do not recall any discussion having taken place concerning comparisons of derived NOEL for histopathology versus the NOEL for plasma cholinesterase inhibition, nor any arguements as to usefulness of a nose-only study. **In my view, one cannot predict what the nose-only study will show regarding effects on the nasal tissues, which needs to be addressed**. Until such work is done, the added 10 UF is called for, as disclosed in the committee "expert's" August 27, 1998 submittal to the committee, and as supported by HED's February 1997 "Toxicology Endpoint Selection Process".

More specific to your comment, the derived NOEL of 0.003 mg/L shown in your document should be given as 0.001 mg/kg according to the committee's decision to employ the 10X uncertainty factor. I Am not certain of the point you attempt to make in contrasting a derived NOEL for nasal effects versus a NOEL for cholinesterase inhibition. Why not versus a derived NOEL for cholinesterase inhibition of 0.0045 mg/L? The contrast would then not seem so remarkable. Yet, the DER claims there was no NOEL for cholinesterase inhibition where concentrations tested were 0, 0.1, 0.45 and 2.01 mg/L. The Agency employs safety factors of 10X from animal to man and another 10X for variations in human sensitivity, so the bulk of the contrast you cite (1500 fold) rests with these legitimate factors universally applied for the protection of the public health.

P. 10, paragraph 4: I do not recall this conclusion as having been reached during any of the three meetings of the HIARC in late August. Am I to conclude from this that these various conclusions with respect to the inhalation study were drawn at a meeting I was not privileged to attend? If so, the date, participants, etc. should be incorporated in this record. In any case you say: "If another study is conducted, it would have to be 'nose-only' exposure in which case the NOEL/LOEL will be higher." Higher than what? There is no NOEL. Further, if you mean the atmospheric concentration eliciting *nasal tissue* effects, it is necessary that you present reference material showing that *nasal tissue* effects as opposed to non-respiratory tissue effects are differentially affected in the two kinds of studies. However, even that would be inadequate since each test material potentially has its unique effects on nasal tissues, and whether there is a systemic component is knowable only on a compound by compound basis. Since this has not been done for malathion, it cannot be presumed to fall one way or the other in the absence of testing, particularly since the effect in the existing study is said to be severe. Logically, and in being consistent with your obligations to protect the public health, further testing should replace presumptive rationalization. It is my understanding that excepting *local* respiratory system effects (as opposed to *systemic*) effects, the whole body assay is conservative and when negative is acceptable. However, when positive, a repeat nose-only study may yield less sever *nasal* effects only if oral ingestion contributes to expression of the effect. So if that is the case, testing by the latter procedure *may*, indeed, get one "off the hook". Nonetheless, when the effect precluding assignment of a NOEL is a respiratory system effect, additional testing is necessary at lower concentrations to identify a NOEL. Until that time, because the effects on nasal tissues are described as sever and occurring in essentially all animals at the LOEL, a 10X as opposed to 3X factor must be imposed. I should remind you this was the recommendation of a consensus of the external reviewers and your committee's designated "expert" at the Augusty 27 meeting. Nothing has changeed since then, at least from my perspective. **Your statement represents a presumption that nasal tissues would be differentially affected in the two kinds of inhalation studies that negates proper end point selection in the face of a glaringly positive finding with no NOEL. That cannot be accepted in lieu of actual data. The purpose of another study would be to identify the NOEL for nasal tissue effects, as this has not been identified in any existing study**. Also, don't forget there was no NOEL for the effects in question after only 2-weeks of testing in the range-finding study, suggesting effects on nasal tissues are of early onset, which should be a weighing factor in your assessment for the need of additional subchronic testing to identify NOELs.

P. 11, paragraph 2: You embolden the last sentence as if to cast aspersions on the appropriatness of the question of whether the chronic toxicity/carcinogenicity study weighs at all in the decision to retain or discount the FQPA imposed 10X safety factor. Well obviously the study makes no distinction between susceptability of young and old animals. However, I am often troubled by statements such as that on your p. 7, paragraph 5, where it is said: "At present the determination of susceptability is made not based on the results of one study (where in fact one *appropriate* study that is positive will do) but rather on a *weight-of-evidence* (emphasis added) basis that includes acute and subchronic neurotoxicity studies, the prental developmental toxicity studies in rats and rabbits, the 2-generation reproduction toxicity study in rats as well as *the toxicity profile of the chemical* (emphasis added). I put this question forward to make it *transparent* to observers that this major study (combined chronic toxicity/carcinogenicity) does not contribute anything magical to the claim of the

weight-of-evidence toward justifying removal of the 10X safety factor for the protection of infants and children. In my view illegitimate mileage is often reaped under the claim "weight-of-evidence" when in fact the well may be rather dry. Where the FQPA 10X factor is concerned, if young and developing individuals are shown to be more sensitive compared to adults in either or both developmental or reproduction studies, the factor remains. In fact, your embolden statement says as much here.

P. 11, paragraph 5: HIARC conclusion notes an *ad hoc* subgroup report of November 13, 1997. There is nothing new here that might serve to overide the recommendations of the external reviewers. In fact it was in part due to my differences of opinion with respect to the conclusions of the *ad hoc* committee that prompted the Agency to invite external toxicologists to vote on these differences of opinion. The external reviewers, with the *ad hoc* committee report before them, in addition to my assessments and the study DERs, confirmed the position advocated in my reviews. Also, I find it regrettable that the HIARC does not even acknowledge, let alone address, additional comments that I, in good faith, submitted to the committee dated January 15, 1998 concerning this subject. Your presenting only the conclusions of the *ad hoc* committee do not afford your reader the benefit of ideas I have brought to this table, which I will not take the time to reiterate here. Nonetheless, my comments and assessment are a part of the record, which I trust will accompany this HIARC report for anyone to see.

P. 12, paragraph 7: Your Panel's Response statement does not adequately embrace the complexities of the comments of the external reviewers. Furthermore, I do not believe it is accurate. I attempted to pull together their conclusions in a paper dated July 27, 1998 submitted to the HIARC Chairman, entitled "Consolidation of External Peer Reviewer's Comments on Malathion non-Cancer Issues, which I trust will be part of the HIARC committee record and fully apparent there. That being the case I will not attempt to suggest revisions to your Panel's Response, but do suggest you revise the statement.

P. 12, paragraph 9: There is nothing *new* offered in citing the *ad hoc* report that serves to compromise the recommendations of the external reviewers, as the *ad hoc* document was submitted to the external reviewers along with my stated objections to the conclusions of the *ad hoc* report, as well as study DERs. In other words, the external reviewers made their recommendations in the face of the *ad hoc* report. I will not take the time here to reiterate my reasons for recommending definitive behavioral effects testing.

P. 13, paragraph 7: The HIARC concluded "..... the entire data base should be examined to see if any peculiarities exist that could serve as a basis for claims of sex-linked sensitivity." I agree with this conclusion and trust there will be follow-up.

P. 14, paragraph 1: In saying that there is no consistent difference in sensitivity of males versus females, you neglected to cite your November 13 *ad hoc* committee report which concluded females were more sensitive. The fundamental question that needs to be address is whether women (girls) are more sensitive than men (boys).

P. 14, paragraph 6: Panel's Report should say in the case of the one member who said no, qualified his no to be applicable as long as the rat study as opposed to the human study serves as the basis for the RfD.

II GENERAL COMMENTS

1) It should be stated somewhere up front in the HIARC report the reason for the external peer review, and exactly what documents were included in the package to that panel of experts, e.g. all malathion DERs, the December 17, 1997 report of the November 6, 1997 HIARC meeting, the November 13, 1997 *ad hoc*

subcommittee report, the bulk of the memoranda I submitted to the committee following the November 6, 1997 meeting and *all* of the questions submitted to the panel. I should note all of this information needs to be publicly accessible

2) It is my observation that the external reviewers' conclusions are in many cases complex and are not adequately captured in the brief statements offered as the "Panel's Response" under the various questions in the HIARC draft document of October 27, 1998. I recommend the "Consolidation of External Peer Reviewer's Comments on Malathion non-Cancer Issues" dated July 27, 1998, which was submitted to the committee, as a preferred assessment of the reviewer's comments. This July 27 document must be available as part of the public record.

3) Following the November 6, 1997 meeting on malathion, I have submitted *in good faith* some fourteen or so memoranda to the committee expressing my scientific concerns over the data base. Although the bulk of these were submitted to the external reviewers, it is particularly disappointing that the HIARC has not responded specifically to these, nor do they find any mention in the HIARC report, even though, by in large, they found favor with the external reviewers, suggesting they have scientific merit. These memoranda must be available as part of the public record of the HIARC meetings to consider malathion.

4) When addressing the question of relative sensitivities of young/developing versus adult animals, I noted at the August meeting that two studies on the one-liners showed the young animals to be more sensitive than adults. These studies were: a) a Guideline 81-1 American Cyanamid Company acute oral study on 95% a.i. malathion in the cow, where reportedly the LD50s were 80 mg/kg (calf) and 560 mg/kg (cow); b) an acute intraperitoneal study in male rats on malathion technical (purity not stated, however in reference to the same published work for this study, Substitue Chemical Program 1975 (p. 66) indicates purity as 99%), where the LD50s were 750 mg/kg (adult) versus 340 mg/kg (weanling). There is no acknowledgement of this in the minutes. Also, the Substitute Chenical Program 1975 says: "Young animals appear to be more susceptible to malathion than older animals (Brodeur and DuBoise, 1963)." (p. 67) Along these same lines, I would mention the following publication: Mendoza, C. E. (1976) Toxicity and Effects of Malathion on Esterases of Suckling Albino Rats., Toxicol. Appl. Pharmacol. 35, 229-238. This particular publication has not, to my knowledge, received a formal review. However, it appears in a recognized peer reviewed journal. Among other conclusions reached in this work, the study claims that one-day-old Wistar rats were found to be *nine times* (close, I might add, to that magical 10X factor imposed by Congress) more susceptible to malathion than seventeen-day-old pups. Accordingly, the LD50 for one-day-old rats as performed repeatedly was 209 (ranging 177-250) mg/kg as compared to LD50 values for seventeen-day-old rats of 1806 (ranging 1415-2003) mg/kg. The test material was identified as American Cyanamid 99.3% a.i. malathion. Such information as this serves to support the evidence of enhanced sensitivity of young rats evident in the Guideline reproduction study and in turn support the 10X safety factor imposed under FQPA.

5) An issue not addressed by the HIARC at its August meeting was that of the response of the external reviewers to the question of the adequacy of the malathion data base. This question was posed among a set of preliminary questions to the external reviewers by HED's external peer review coordinator, and I recommended in an August 17, 1998 memorandum to the committee chairman that it be discussed. In essence, the external reviewers identify several data gaps or data deficiencies which are summarized in this August 17 letter. Now whether these deficiencies are data gaps in the strict sense of being unsatisfied end points in Guideline studies (as I believe some are), or inadequacies in the overall assessment of malathion to address health effects concerns, is probably one more of semantics than substance with respect to the intent of Congress to protect infants and children. A most noteable statement along these lines was made by Dr. Dourson, who wrote: *"I am not satisfied that the potential risk to humans is addressed with the data available in this review package." (P. 3 of his June 3, 1998 comments).* **So the point I am making here is that it**

cannot be claimed by HIARC that the no-data-gap qualifier required under FQPA for removal of the 10X safety factor has been met.

6) For the most part, the HIARC has used the same reasoning employed in November 1997 to refute the conclusions/recommendations of the expert panel. There is little evidence the HIARC has been influenced by the external reviewers, whose task it was to weigh in on the differences of opinion between myself and the committee. It is not altogether clear to me why the issues were referred back to the HIARC, but in any case, all of the committee's decisions require review and confirmation outside HED before they become regulatory acceptable. **The following particularly important conclusions are supported by *at least* a consensus of the external reviewers who had the full package of data in hand:**

a) An acute (one-day) end point as high as 0.50 mg/kg is not supported by the data base. It is particularly important this be addressed if the acute (one-day) end point finds use in risk assessments for exposures of up to 7 days;

b) In the absence of assessments of cholinesterase inhibition in young/developing animals versus older animals in developmental and reproduction studies, and the absence of behavioral effects testing in reproduction studies it cannot be interpreted that such studies provide the *reliable* information (as required by Congress) of no increased sensitivity of young animals necessary to discount the 10X safety factor imposed under FQPA for the protection of infants and children. To the extent these studies do not satisfy as *reliable*, the removal of the 10X safety factor imposed under FQPA is not defensible.

c) The actual finding of increased sensitivity of pups versus adults in the reproduction study confirms retention of the 10X safety factor imposed under FQPA for the protection of infants and children (note: I assert an opinion here that a clear consensus among external reviewers would have been expressed in support of this had they been aware that malathion has not been found in milk and that adult animals in the reproduction study were not affected at any dose level, while pup body weight gains were compromized at both the high dose and penultimate dose levels in this study. In further support of a finding that young individuals are more sensitive than older animals to malathion are three LD50 studies cited above showing greater sensitivity of the young. The external reviewers may not have known of these additional studies). Again, in view of the actual findings of enhanced sensitivity of the young, the removal of the 10X safety factor imposed under FQPA would be illegitimate.

d) Given the evidence of a post 3 months recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, 50 ppm cannot be concluded to have been a NOEL for the first three months of testing, which is a considerable time frame. In view of this, there is no NOEL for cholinesterase inhibition for females in this study, and hence, in the absence of any additional uncertainty factor, it cannot serve as the basis for the RfD.

e) Cholinesterase methodology may be a problem in this study which needs to be addressed.

f) A shift from the human study to the rat study as the basis for the RfD is unsupported.

g) Use of a mere 10X safety factor to allow for "uncertainties" (knowing of the lack of carboxylesterase in human plasma) in interspecies variability is held to be inadequate should the rat study supplant the human study.

h) The uncertainty factor to be applied to the inhalation end points (intermediate and long term) to compensate for the absence of a NOEL for nasal and laryngal degeneration/hyperplasia is 10X.

i) A consensus exists among external reviewers that additional assessment of some sort is indicated to address the absence of NOELs in the inhalation study.

j) Retinal tissue histopathology slides should be submitted for independent pathology assessment as called for in the study DER, and retinal tissues slides not taken from lower dose group animals should be submitted, according to Guideline requirements.

k) Additional behavioral effects testing, e.g. developmental neurotoxicity, should be required for malathion as is being done for certain other cholinesterase inhibiting pesticides.

l) Additional testing in animal models should be required to quantitate any gender specific disparity with respect to cholinesterase inhibition.

Brian Dementi, Ph.D., DABT
Toxicologist
Health Effects Division