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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR No. 0052129

MEMORANDUM

DATE: November 9, 2004

SUBJECT: D292658: Malathion (057701) Amendment 1 to comparative cholinesterase inhibition study in adult and juvenile rats (MRID 46005001)

TO: Thomas Moriarty
Chemical Review Manager
Special Review and Reregistration Division (7508C)

FROM: Brian Dementi, Ph.D., D.A.B.T. Brian Dementi 11/9/04
Toxicology Branch
Health Effects Division (7509C)

and

Susan Makris Susan T Makris
Toxicology Branch
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THRU: Alberto Protzel, Ph.D., Branch Senior Scientist Alberto Protzel
Toxicology Branch
Health Effects Division (7509C)

CC: William Wooge (7509C)

ACTION REQUESTED: Review an amendment (MRID 46005001) to the final comparative cholinesterase study in adult and juvenile rats with malathion (MRID 45566201), a companion study to the developmental neurotoxicity study in rats (MRID 45646401):

CONCLUSION: The amended study report pages do not change the Agency's interpretation of the submitted study nor alter the final conclusion. An executive summary is presented below:

Citation: Fulcher, S.M.. (2003) Amendment 1 to the final report; Malathion: effects on cholinesterase in the CD rat (adult and juvenile) by oral gavage

administration. Huntingdon Life Sciences, Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. Doc. No. CHV067/012452. June 19, 2003. MRID 46005001. Unpublished

Amendment to: Fulcher, S.M.. (2001) Malathion: Effects on cholinesterase in the CD rat (adult and juvenile) by oral gavage administration. Huntingdon Life Sciences, Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. Doc. No. CHV067/012452. November 30, 2001. MRID 45566201. Unpublished

Executive Summary: An Amendment (MRID 46005001) to a special cholinesterase study (45566201) of malathion in rats, plus the study itself, have been evaluated by the Agency concerning study design and statistical analysis of the data.

In the Agency's opinion, the decision by the authors to exclude outliers, in the absence of laboratory problems, based solely on results of statistical tests is inappropriate. Particularly, when so few animals as eight per group were tested. With so few animals per group, it may well be that the one animal perceived to be an outlier, in this case in the PND11 control group, could be indeed truly representative of certain members of the population. This aspect of too few animals per group constitutes an inherent weakness in the study when focusing upon a relatively small but critical difference in cholinesterase activity between groups. The Agency is expected to be conservative in its decisions, such that the incumbency resides with the registrant to test more definitively in this case at this critical dose level in order to distinguish NOAEL from an effect level.

Concerning statistical treatment of the data, an Agency statistician, having considered the original and amended statistical procedures, concluded that no advantage derives from the amended approach. Basically he finds that the original statistical treatment yields all that can be expected, statistically, given the small number of animals (eight/group) in the study.

The cholinesterase activity measures following acute or repeated gavage doses of malathion in this study [summarized in the original review of the study, MRID 45566201], demonstrate that juvenile rats **are more** susceptible than adults. Overall, this susceptibility was observed in terms of the dose level at which effects were observed (i.e., the NOAELs for cholinesterase inhibition were lower for juveniles than for adults), and the compartments in which a response was elicited [e.g., brain cholinesterase was inhibited in offspring (PND 11) in the acute study at doses as low as 50 mg/kg, but was not observed in adults up to the highest dose tested, 450 mg/kg; a comparison illustrating an enhanced offspring versus adult susceptibility of this CNS enzyme of over 90-fold]. Also, the magnitude of the response (i.e., when inhibition was noted for both age groups at the same dose level, the percent inhibition was substantially greater for pups than for young adults). In the acute study, the magnitude of brain cholinesterase inhibition in offspring was 81-84% at 450 mg/kg, while no inhibition of the CNS enzyme was observed in adults following a single dose of 450 mg/kg.

In the study involving repeated exposures, data are not available at doses above the 150 mg/kg/day level that would be expected to have permitted a better estimate of offspring versus

adult susceptibility. Hence, for the moment, the acute study most reliably serves among all malathion studies in the identification of the offspring versus adult FQPA susceptibility factor as is required under the Food Quality Protection Act (FQPA).

This same susceptibility was not demonstrated for GD 20 fetuses when compared to dams, following maternal exposure from GD 6-20, suggesting little or no placental transfer of cholinesterase inhibiting compound(s) following malathion administration to pregnant dams.

It is concluded that the submitted amendment (MRID 46005001) to the original study report (MRID 45566201) does not change the Agency's interpretation of the submitted study nor alters the final conclusion. This study is classified **acceptable/nonguideline** for the determination of plasma, RBC, and brain cholinesterase activities following treatment with malathion in adult, fetal, and juvenile rats.

Data review:

The Registrant, Cheminova, submitted an amendment to the final report on the effects of cholinesterase in the adult and juvenile rat following oral gavage administration of malathion. The changes to the final report were detailed as follows (pp. 5-6):

Details of amendment	Reason for amendment
Replacement page 1: Total number of pages amended. Replacement page 5: Contents list page numbers amended, addendum 6 added. Replacement pages 11-13: Test modified to reflect results of statistical review. Overall interpretation not altered. Replacement pages 15-17: Tabular summary amended to reflect new statistical analysis for erythrocyte activity.	The statistical test used to analyse the erythrocyte cholinesterase data was reviewed with particular attention being paid to outliers and the possibilities transforming the data before analysis. The review established that a more accurate statistical analysis could be achieved by excluding outlying values and transforming the data before performing intergroup tests. The results of this reanalysis have been incorporated into the report in the form of replacement pages that can be inserted into the original report.

Replacement pages 35-36: Details of statistical test employed modified to include new statistical analysis. Replacement pages 41-42: Results modified to reflect statistical review. Overall interpretation unaltered. Replacement pages 44: Additional references added. Replacement pages 99-112: Table amended to include new statistical analysis for erythrocyte cholinesterase activity. Replacement pages 239, 240, and 261: Appendices amended to indicate outlier values excluded from group mean values. Replacement pages 296-300: Addendum 6 (Review of the statistical analysis of erythrocyte cholinesterase) added. Replacement pages 301-344: Page number corrected (due to addition of Addendum 6)	Additionally actual p-values have replaced the normal indicators at the 1% and 5% level for the cholinesterase data table (with the exception of the tabular summary).

The changes made to the report, and the Agency's review of these changes, are discussed below:

The focal points of Cheminova's re-assessment of the cholinesterase data reside with the LOAELs/NOAELs for erythrocyte cholinesterase inhibition among offspring in the PND 11 acute single dosing study and the PND 21 repeated dosing studies.

Comparative inspection of findings for the PND 11 acute single dose and PND 21 repeated dose studies (offspring and adult) in the respective "Tabular Summaries" in the original study submission (MRID 45566201) and the amended report (MRID 46005000), pp. 16-17 in both reports, reveals revisions in the amended report only to the erythrocyte cholinesterase data and only among male offspring. All other findings, for both offspring and adult animals remain the same in these reports.

In the case of the PND 11 acute study, the statistically significant 16% inhibition noted for males at the lowest dose of 5 mg/kg ($p < 0.05$, Table 37, p. 103) in the original submission appears as an 11%, non-statistically significant finding ($p = 0.078$, Table 37, p. 103) in the amended report. The registrant considers this revision to have changed the LOAEL for the study from 5 mg/kg to 50 mg/kg, a critical end point for the study. It should be noted that at the 50, 150 and 450 mg/kg dose levels, the reported inhibitions of erythrocyte cholinesterase among males are likewise less in the amended report, but these are of comparatively minor importance. These changes in inhibition for male PND 11 offspring erythrocyte cholinesterase derive from the registrant's, or study director's, removal from the control data set one animal (# 8101) considered to be an outlier based upon application of "A two-tailed Dixon-type test for outliers (HLS 2003).....". (p. 36) It also derives in part from a preferred statistical treatment of the data in the amended report. While the statistical treatment of the data is somewhat esoteric, in MRID 45941901 the registrant says: "For its initial analyses of these data, HLS conducted a Williams' test for all of the cholinesterase comparisons. The Williams' test is a multiple dose comparison test that is used when there is a monotonic dose-response. For comparison with a single dose

group, Williams' test reduces to a simple t-test (Student's t-test). Therefore, for its initial analysis, HLS essentially used a simple t-test for statistical comparisons at the 5 mg/kg bw dose group (the lowest dose group). Cheminova believes that a simple t-test is not the best test for group comparisons with unequal variances. In this case, a Welch's modified t-test is the most appropriate test. Welch's modified t-test is commonly used, and is actually built into the t-test procedure in software programs such as Microsoft Excel (R) and S-Plus when one specifies that the variances are unequal." (pp. 15-16). Cheminova goes on to explain that based upon Bartlett's test, "HLS used a pooled variance between all the dose groups for all of its comparison tests", while "Cheminova believes that the best estimate of variance for the comparisons with the 5 mg/kg bw group is the pooled variance between the control group and the 5 mg/kg bw dose group." (p. 16)

The questions to be addressed are 1) the appropriateness of removing an animal considered to be an outlier and 2) whether the statistical approach taken in the original study was inappropriate, or less definitive than other statistical approaches.

In the Agency's opinion, it is not appropriate to remove a data point perceived to be an outlier, particularly when so few animals as eight per group were tested. With so few animals per group, it may well be that the one animal perceived to be an outlier, in this case in the PND11 control group, could be indeed truly representative of certain members of the population. This aspect of too few animals per group constitutes an inherent weakness in the study when focusing upon a relatively small but critical difference in cholinesterase activity between groups. The Agency is expected to be conservative in its decisions, such that the incumbency resides with the registrant to test more definitively in this case at this critical dose level in order to distinguish NOAEL from an effect level.

For the sake of discussion, as it may relate to this perspective on outliers, the amended report reads in describing clinical signs following the 450 mg/kg dose in the PND 11 study: "A single administration to PND 11 offspring was associated with body tremors 1-2 hours after dosing in 5/16 animals and a further offspring was found in a moribund state and killed 1 hour after dosing. Necropsy findings were unremarkable." (p. 11) Presumably 10/16 animals were asymptomatic. Are we to assume that the one moribund animal was an outlier, and discount it from consideration as evidence of an effect of malathion? There are not enough animals to make that conclusion either. Was this an unusually susceptible animal, but nonetheless possibly representative of others in the larger population?

Further along in the registrant's text, the registrant presents a revised statistical treatment of the data (pp. 12-22), which treatment also discounts as meaningful any difference between the control and 5 mg/kg/day groups in the PND11 study. An Agency statistician, having considered the original and amended statistical procedures, concluded that no advantage derives from the amended approach. Basically he finds that the original statistical treatment yields all that can be expected, statistically, given the small number of animals (eight/group) in the study.

The report of EPA's statistician, Dr. Leonid Kopylev, is appended to this report.

As it concerns the PND 21 repeated dose study, a revised statistical treatment of the data was

similarly employed: "We also reevaluated the data for cholinesterase activity for PND 21 offspring using the same procedures that we used for our reanalysis of these data for PND 11 offspring. The results of our reanalysis of the data for PND 21 male offspring are presented below in Table 6." (MRID 45941901, p. 20). While not altering any of the cholinesterase data or percentage inhibitions for dose groups as revealed in the original study and amended report "Tabular Summaries", this reanalysis did alter the statistical interpretation for the 5 mg/kg/day male dose group's 17% erythrocyte cholinesterase inhibition from one of being statistically significant ($p < 0.05$, Table 39, p. 107) in the original submission to non-significant ($p = 0.067$, Table 39, p. 107) in the amended report. Hence, for the PND 21 repeated dosing study, the LOAEL for offspring becomes 50 mg/kg/day according to the amended submission, i.e. in the absence of statistical significance, the registrant discounts the 17% inhibition as an effect level. However, the Agency concludes that inhibition of 17% cannot be discounted even though statistical significance did not quite achieve the $p = 0.05$ criterion of significance. Furthermore, as in the case of the PND 11 acute study, the Agency statistician reports no advantage with the amended statistical approach, and, again, for the same reason, too few animals per dose group.

The report of EPA's statistician, Dr. Leonid Kopylev, is appended to this report.

While we disagree with the registrant's removal of an outlier from the control group in the PND 11 acute study, which diminished the low dose group level of inhibition from 17% to 11%, it is noteworthy that a similar consideration of the PND 21 individual data discloses a possible "outlier" (or inordinately high value) (# 3503) in the 5 mg/kg/day female group, which if deleted would increase the percentage inhibition for this group from 15% as reportedly non-significant to 23% inhibition, which would likely be statistically significant, thus enhancing the conclusion that 5 mg/kg/day was an effect level in the PND 21 repeated dose study.

So, while we do not favor deleting animals from among so few as eight/group on the grounds of their being outliers for the reasons stated previously, if pursued nonetheless in this study it may work both ways.

The amendment (p. 298) identified two additional outliers (PND 4 high dose female and PND 60 high dose male) neither of which when excluded from the cohort altered the study interpretation.

Conclusion: The submitted amendment (MRID 46005001) to the original study report (MRID 45566201) does not change the Agency's interpretation of the submitted study nor alter the final conclusion.

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

OFFICE OF WATER

MEMORANDUM

DATE: June 25, 2004

TO: Susan Makris, M.S., Acting Branch Chief
Brian Dementi, Ph.D., DABT
Toxicology Branch
Health Effects Division
Office of Pesticide Programs

FROM: Leonid Kopylev, Mathematical Statistician
Engineering and Analysis Division
Office of Science and Technology

SUBJECT: Design and statistical analysis of cholinesterase measures from the malathion range-finding and comparative cholinesterase studies in rats.

This memo responds to your request for a statistical review of the two studies submitted by Cheminova A/S. It addresses two deficiencies in the design and statistical analysis of both the range-finding and comparative cholinesterase studies by Cheminova. These deficiencies are: 1) inadequate number of animals in each group in the comparative cholinesterase studies resulting in low statistical power of the tests and 2) inappropriate treatment of outliers.

The authors of the comparative cholinesterase study chose sample sizes that are more than 2 times less than sample sizes in the range-finding study although variability measured in the range-finding study indicated that sample sizes that are comparable to those used in the range-finding study should have been used in the comparative cholinesterase study. Because of that, details of statistical methodology used by the comparative cholinesterase study authors are not considered in this memo, as no statistical methodology would perform well for selected small sample sizes in the presence of the variability observed in the data.

As a consequence of selected sample sizes, the statistical power of the tests is below 50% for regulatory important comparison. Because of the small sample size chosen for the comparative cholinesterase study and resulting low power of statistical comparisons of group means, the absence of statistical significance is not evidence to conclude that the null hypothesis of no

difference between means is accepted. In such a situation, the evaluation of biological significance becomes critical to the interpretation of treatment-related effects.

The decision by authors to exclude outliers based solely on results of statistical tests is inappropriate. The numbers should be retained and used to calculate group means.

1. Range-finding study and design of the comparative cholinesterase study; sample size and statistical power issues.

The range-finding study and comparative cholinesterase studies with malathion were submitted to the Agency by the registrant, Cheminova, in partial compliance with the requirements of a Data-Call-In that was issued by the Office of Pesticide Programs in 1999. Executive Summaries of these studies are appended to this memo as Attachments A and B.

The range-finding study involved 5 groups (control and 4 treatment groups) of PND21 male and female rat pups (Table 11 of range-finding DER). Each of the groups contained from 18 to 20 animals. The treatment groups were administered 7.5, 35, 75 and 150 mg/kg of Malathion per day and monotonically increasing inhibition with increased dose was observed. The choice of the doses did not correspond to the doses chosen for the effects study except for the highest dose, 150 mg/kg/day group. Because the smallest dose group is where regulatory interest most times is, the minimum sample size calculations for the comparative cholinesterase study had to be based on the control group. Based on standard statistical methods for 2-sample tests, one could estimate how many rats in each group would be needed. With variability measured during the range-finding study for Plasma, RBC and Brain measurements, the following number of animals was required to detect a biologically significant 15% difference between control and treatment group at the 0.05 significance level:

Power	Number of animals in each group	
	Control	Treatment
	Male animals	
80%	18	18
90%	24	24
	Female animals	
80%	12	12
90%	16	16

It was imperative that at least 18 male animals and 12 female animals would be used for the control and 5 mg/kg/day groups for PND21 and all other animal groups where variability is similar. When a larger difference is expected (as for the 150 mg/kg/day group) a smaller number of animals could have been chosen.

Using the same statistical methods, one could calculate that the sample size chosen for the effects study (n=8) would provide statistical power of only about 47% for males and 65% for females. Similarly, from tables 5a and 5b of the comparative cholinesterase study DER, one could verify

that the power of the t-test comparing RBC measurement in the 5 mg/kg/day group with the control group is only 46% for PND11 males and is only 26% for PND21 females and 44% for PND21 males (in all these situations, the difference between means exceeded the 15% cholinesterase inhibition that was considered to be biologically significant by Agency scientists). That means that the statistical test used by Cheminova had little chance to detect a significant effect.

2. Treatment of outliers.

The study authors chose to exclude several observations that they considered to be outliers based solely on the results of a statistical test for outliers. While this approach can be useful in identifying extremely large or extremely small measurement values that require additional review, it is not appropriate to remove them solely on the basis of a statistical procedure. Because extreme values can be expected to occur on occasion, due to the variability in animals, a review of the laboratory records associated with the measurement may establish whether the extreme value was caused by failure to follow proper laboratory procedures. If the laboratory failed to analyze the results properly, it may be appropriate to exclude the measurement result from the statistical analysis. In the absence of laboratory problems, outliers should not be excluded based solely on statistical tests.

Furthermore, excluding values from small sample sizes is especially problematic, because of the uncertainty about underlying distribution. Before excluding such values from small sample sizes, statisticians generally perform sensitivity analyses to determine the impact on the results of including or excluding these values.

Attachment A – Range-finding Study

CITATION: Fulcher, S.M. (2002) Malathion dose finding study in CD rats by oral gavage administration preliminary to developmental neurotoxicity study. Huntingdon Life Sciences, Ltd., Cambridgeshire PE28 4HS, England. Laboratory Project No. CHV/062, February 27, 2002. MRID 45627001. Unpublished

EXECUTIVE SUMMARY: A preliminary dose range-finding developmental neurotoxicity study (MRID 45627001) with malathion (96% a.i., batch/lot 9010501) was conducted in two phases. In Phase 1, malathion was administered by gavage to 15 female Crl:CD® BR rats per dose at dose levels of 0, 7.5, 750 or 1250 mg/kg bw/day. Ten maternal animals/group were administered the test substance from gestation day (GD) 6 through postnatal day (PND) 10; an additional five dams/group were dosed on GD 6-20. Following mortalities at 1250 mg/kg/day during the first four days of treatment, the dose for this group was reduced to 1000 mg/kg/day. In Phase 2, 10 maternal animals/group were administered the test substance from GD 6 through PND 10; an additional five dams/group were dosed on GD 6-20, at doses of 0, 7.5, 35, 75, or 150 mg/kg/day. In both phases, two male and two female pups/litter were treated from PND 11 to 21. For Phase 1, an additional 2 male and 2 female pups/litter (from dams treated at 0 or 7.5 mg/kg/day) were also dosed from PND 11 to 21 at 200 or 450 mg/kg/day. The females treated up to GD 20 were killed three hours after dosing on that day; litter data were assessed and cholinesterase activity determined in maternal and fetal plasma, RBC, and brain. Treated offspring were killed two hours after dosing on postnatal day 21 and cholinesterase activities determined.

Under the conditions of this study, no adverse effects of treatment were observed in maternal animals at 7.5 or 35 mg/kg/day. Transient post-dosing salivation was seen in the majority of dams at 75 and 150 mg/kg/day. Signs of severe toxicity were observed at 750 and 1250/1000 mg/kg/day, and included tremors, prostrate posture, abnormal gait, decreased body weight and food consumption, moribundity, and mortality; dosing was stopped for these groups and survivors were sacrificed on GD 20. At GD 20, RBC cholinesterase inhibition was observed in dams at 75 mg/kg/day and above; plasma and brain cholineserase inhibition were observed at 750 mg/kg/day and above.

In offspring that were dosed directly, overt clinical signs of toxicity (body tremors and moribundity) were observed at doses of 200 and 450 mg/kg/day; due to the excessive toxicity dosing was terminated and pups sacrificed before reaching weaning. RBC cholinesterase inhibition was observed at all doses tested (i.e., 7.5 mg/kg/day and above) in PND 21 pups. Brain cholinesterase inhibition was seen at 75 mg/kg/day and above, and plasma cholinesterase was inhibited at 150 mg/kg/day and above. For GD 20 fetuses, RBC cholinesterase was inhibited at 750 mg/kg/day and above.

The results from this study were used to select the doses used in the definitive developmental neurotoxicity study (MRID 45646401). The highest dose tested in that study was set at 150 mg/kg/day, based upon the severity of clinical signs noted at 200 mg/kg/day in directly dosed pups on this dose range-finding study.

Attachment B – Comparative Cholinesterase Study

CITATION: Fulcher, S.M. (2001) Malathion: Effects on cholinesterase in the CD rat (adult and juvenile) by oral gavage administration. Huntingdon Life Sciences, Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. Doc. No. CHV067/012452. November 30, 2001. MRID 45566201. Unpublished

EXECUTIVE SUMMARY: In a special comparative cholinesterase study (MRID 45566201), malathion (96.0% a.i., batch/lot # 9010501) was administered to groups of Crl:CD® (SD) IGS BR rats by gavage at dose levels of 0, 5, 50, 150, or 450 mg/kg bw/day for acute exposures and 0, 5, 50, and 150 mg/kg/day for repeated exposures. Treatment groups consisted of 9 pregnant dams treated from GD 6 through GD 20 and terminated; 10 pregnant dams treated from GD 6 through PND 10 followed by treatment of 1 male and 1 female offspring/litter on PND 11 through PND 21; and groups of 8 untreated dams whose offspring were treated on PND 11. In addition, groups of 16 adult male and female rats were given either a single dose or 11 consecutive days of dosing with malathion. The primary purpose of this study was to determine the effect of malathion on blood and brain cholinesterase activities in adult male and female rats, pregnant dams, fetuses, and juvenile rats following both acute and repeated exposures.

An acute 450 mg/kg dose of malathion resulted in tremors in 5 of 16 PND 11 pups at 1-2 hours post-treatment, as well as moribundity in one pup; no clinical observations were noted in young adults at this dose. Repeated doses of malathion resulted in post-dose salivation at 150 mg/kg/day in dams during gestation and/or lactation, but did not adversely affect survival, clinical observations, body weight, body weight gain, brain weight, or gross pathology in adult male and female rats, juveniles, or fetuses. Additionally, reproductive performance, gestation length, sex ratio, pre- and postnatal viability were unaffected.

However, acute or repeated exposure to malathion resulted in statistically and biologically significant decreases in cholinesterase activity in the blood and/or brain in dams, fetuses, weanling pups, and adult male and female rats. In pups, effects were noted at 5 mg/kg in males and 50 mg/kg in females following single dose acute exposures, and at 5 mg/kg/day in both sexes after repeated exposures. Following a single dose to young adults, effects were observed at 450 mg/kg, while after 11 or 14 doses, effects were observed at 50 mg/kg/day in young adults and pregnant dams. In pups, effects were noted at 5 mg/kg/day in males and 50 mg/kg/day in females following single dose acute exposures, and at 5 mg/kg/day in both sexes after repeated exposures. By PND 60 (39 days after the last dose), cholinesterase activity levels in offspring were similar between control and treated groups.

For acute exposures:

the adult LOAEL for brain ChEI is >450 mg/kg (both sexes)
the adult NOAEL for brain ChEI is ≥450 mg/kg;

the offspring LOAEL for brain ChEI is 50 mg/kg (for males), 150 mg/kg (for females)
the offspring NOAEL for brain ChEI is 5 mg/kg (for males), 50 mg/kg (for females);

the adult LOAEL for red blood cell ChEI is 450 mg/kg (both sexes)
the adult NOAEL for red blood cell ChEI is 150 mg/kg;

the offspring LOAEL for red blood cell ChEI is 5 mg/kg (for males), 50 mg/kg for females
the offspring NOAEL for red blood cell ChEI is <5 mg/kg (for males), 5 mg/kg for females;

the adult LOAEL for plasma ChEI is 450 mg/kg (for males), >450 mg/kg (for females)
the adult NOAEL for plasma ChEI is 150 mg/kg (for males), ≥450 mg/kg (for females);

the offspring LOAEL for plasma ChEI is 50 mg/kg (both sexes)
the offspring NOAEL for plasma ChEI is 5 mg/kg.

For acute exposures, the overall adult LOAEL for cholinesterase inhibition is 450 mg/kg/day for plasma and red blood cells; the adult NOAEL is 150 mg/kg/day.

For acute exposures, the overall offspring LOAEL for cholinesterase inhibition is 5 mg/kg/day for red blood cells; the offspring NOAEL was not determined (<5 mg/kg/day).

For repeated exposures:

the adult LOAEL for brain ChEI is >150 mg/kg (both sexes)
the adult NOAEL for brain ChEI is ≥150 mg/kg;

the offspring LOAEL for brain ChEI is 150 mg/kg (both sexes)
the offspring NOAEL for brain ChEI is 50 mg/kg;

the adult LOAEL for red blood cell ChEI is 50 mg/kg (both sexes)
the adult NOAEL for red blood cell ChEI is 5 mg/kg;
the offspring LOAEL for red blood cell ChEI is 5 mg/kg (both sexes)
the offspring NOAEL for red blood cell ChEI is <5 mg/kg;

the adult LOAEL for plasma ChEI is >150 mg/kg (both sexes)
the adult NOAEL for plasma ChEI is ≥150 mg/kg;

the offspring LOAEL for plasma ChEI is 50 mg/kg (both sexes)
the offspring NOAEL for plasma ChEI is 5 mg/kg.

For repeated exposures, the overall adult LOAEL for cholinesterase inhibition is 50 mg/kg/day for red blood cells; the adult NOAEL is 5 mg/kg/day.

For repeated exposures, the overall offspring LOAEL for cholinesterase inhibition is 5 mg/kg/day for red blood cells; the offspring NOAEL was not determined (<5 mg/kg/day).

The cholinesterase activity measures following acute or repeated gavage doses of malathion in this study, demonstrate that juvenile rats are more susceptible than adults. Overall, this susceptibility was observed in terms of the dose level at which effects were observed (i.e., the

NOAELs for cholinesterase inhibition were lower for juveniles than for adults), the compartments in which a response was elicited (e.g., brain cholinesterase was inhibited in offspring but was not observed in adults up to the highest dose tested), and the magnitude of the response (i.e., when inhibition was noted for both age groups at the same dose level, the percent inhibition was substantially greater for pups than for young adults). This same susceptibility was not demonstrated for GD 20 fetuses when compared to dams, following maternal exposure from GD 6-20.

13544

R103956

Chemical:	**Malathion**
PC Code:	**057701**
***HED* File Code**	**13000 Tox Reviews**
Memo Date:	**11/09/2004**
File ID:	**TX0052129**
Accession Number:	**412-05-0035**

HED Records Reference Center
01/27/2005