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

**OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES**

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

**MEMORANDUM**

March 30, 2007
TXR # 0054537

**SUBJECT:** Data Evaluation Record (DER) for Special Study: Repeat Dose Comparative Cholinesterase Study of Malathion and Malaoxon in Juvenile Rats

**PC Code:** 057701
**DP Barcode:** D332783

**FROM:** Gerome Burke, Ph.D.
Toxicology Branch
Health Effects Division (7509P)

**TO:** William Hazel, Ph.D.
Chief, Reregistration Branch 2
Health Effects Division (7509P)

**THROUGH:** Louis Scarano, P.h.D.
Chief, Toxicology Branch
Health Effects Division (7509P)

## I. CONCLUSIONS

Attached is the Data Evaluation Record (DER) for the above-named study. Rat pups were dosed via the oral (gavage) route with either malathion (0, 5, 25, 50, 150 mg/kg/day) or malaoxon (0, 0.1, 1, 2.5, 4 mg/kg/day) daily for 10 days (postnatal days 11-21). At that time, they were sacrificed and acetylcholinesterase (AChE) activity was measured in the blood (red blood cell or RBC) and brain. EPA performed a benchmark dose (BMD) analysis on these data and calculated the following points of departure for each chemical: 9 mg/kg/day for malathion and 0.3 mg/kg/day for malaoxon. Both are BMDL10 values (the lower bound on the 95$^{th}$ percentile calculation for a 10% decrease in RBC AChE).



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2007

**EPA Reviewer: Gerome V. Burke, Ph.D.**
**Toxicology Branch, Health Effects Division (7509C)**
Signature
3/30/07 Date
**EPA Secondary Reviewer: Louis Scarano, Ph.D.**
**Toxicology Branch, Health Effects Division (7509C)**
Signature
3/30/07 Date

# DATA EVALUATION RECORD

**STUDY TYPE:** Special Study, Effects on Cholinesterase in Neonatal Rats

**PC CODE:** 057701

**DP BARCODE:** D332783
**TXR #:** 0054537

**TEST MATERIAL (PURITY):** Malathion (96% a.i.)

**SYNONYMS:** Fyfanon Technical

**CITATIONS:** Barnett, John F. (2006).Oral (Gavage) Repeat Dose Comparative Cholinesterase Study of Malathion and Malaoxon in Juvenile Rats. Charles River Laboratories, Preclinical Services, Horsham, PA, Charles River Laboratories Preclinical Services Protocol Number: TQC00013. April 21, 2006. MRID 46822201. Unpublished.

**SPONSOR:** Cheminova A/S (EPA Company No. 4787), P.O. Box 9, DK-7620 Lemvig, Denmark

**EXECUTIVE SUMMARY:** In comparative cholinesterase inhibition (ChEI) studies, Malathion (96% a.i., Lot No. 9010501) and Malaoxon (97.7% a.i., Lot No. 849-BSE42C) were administered by gavage to groups of Crl:CD®(SD) rats. Twelve rats/sex/dose were gavaged with malathion at 0 (Vehicle), 5, 25, 50, and 150 mg/kg/day or with malaoxon at 0 (Vehicle), 0.1, 1, 2.5, and 4 mg/kg/day. The test substances in the vehicle (corn oil) or the vehicle alone were administered once daily to the pups on postnatal days (PND) 11-21. The dose volume was 5 ml/kg for both test substances and the vehicle. Blood samples were collected from each of the pups following decapitation and the brains were removed at the end of the study. The samples were collected at 2 hours post-dose for the malathion dose groups and 30 minutes post-dose for the malaoxon dose groups. These blood and brain samples were analyzed for cholinesterase levels.

At 150 mg/kg/day, both male and female pups administered malathion were observed with adverse clinical signs: tremors in the head or whole body (M/F), decreased motor activity (M), impaired righting reflex and splayed forelimbs (M), and pale extremities (F). No test substance-related mortality, effects on body weights, or body weight gains were observed in either the malathion or malaoxon dose groups. Statistically significant reductions in red blood cell (RBC) cholinesterase levels were observed in the 25, 50 and 150 mg/kg/day dose groups (M/F) administered malathion, and the 1, 2.5 and 4 mg/kg/day dose groups (M/F) pups administered malaoxon. Brain cholinesterase levels were statistically significantly reduced only in the 150 mg/kg/day dose group (M/F) administered malathion. There were no statistically significant differences in brain cholinesterase levels (M/F) for pups administered malaoxon.

The registrant submitted a benchmark dose analysis (BMD) (MRID 46821703) for RBC and brain ChE inhibition on this study. The Agency also ran a BMD (Appendix II) to determine the point of departure (POD).

**Based on a BMD analysis of the RBC cholinesterase data, the point of departure is 9 mg/kg/day ($BMDL_{10}$) for Malathion and 0.3 mg/kg/day ($BMDL_{10}$) for Malaoxon.**

These studies are classified **Acceptable/Nonguideline** for the determination of RBC and brain cholinesterase activities following treatment with repeated doses of malathion technical in adult rats and pups.

**COMPLIANCE**: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided for all studies.

## I. MATERIALS AND METHODS:

### A. MATERIALS:

**1. Test material:** Malathion

| | |
|---|---|
| **Description:** | Clear pale yellowish liquid |
| **Lot/Batch #:** | LOT No. 9010501 |
| **Purity:** | 96 % a.i. (Certificate of analysis not provided) |
| **Compound Stability:** | Not provided |
| **CAS # of TGAI:** | 121-75-5 |

**2. Test material:** Malaoxon

| | |
|---|---|
| **Description:** | Clear, colorless liquid |
| **Lot/Batch #:** | LOT No. 849-BSE-42C |
| **Purity:** | 97.7 % a.i. |
| **Compound Stability:** | Not provided |
| **CAS # of TGAI:** | 1634-78-2 |

**2. Vehicle and/or positive control:** Corn oil (CAS No. 8001-30-7, Lot No. 065K0077, Sigma-Aldrich) was used as the vehicle in all studies. No positive control was used.

**3. Test animals (P):**

| | |
|---|---|
| **Species:** | *Rat* |
| **Strain:** | Crl:CD®(Sprague Dawley) |
| **Age and wt. at study initiation:** | The dams were bred at the supplier's facility with male rats of the same source and strain. The dams delivered the pups at the supplier and shipped to arrive at the testing facility on PNDs 4 - 5, 7, and 8. Body weights for the dams were not provided.<br><br>F1 Generation pups (M/F) arrived at the testing facility in two shipments of 120 pups each (120M/120F). In shipment 1, the pups were 4-5 days old and weighed 14.3-29.8g. In shipment 2, the pups were 7-8 days old and weighed 15.8-24.1g. On PND 9 or 10, 24 litters of ten pups per litter (5M/5F) were randomly assigned in the study. |
| **Source:** | Charles River Laboratories, Raleigh, NC |
| **Housing:** | Each dam with a litter of male and female pups was housed in a common nesting box during the postpartum period. |
| **Diet:** | Certified Rodent Diet® #5002, PMI® International, *ad libitum* |
| **Water:** | Tap water filtered through a reverse osmosis membrane was available *ad libitum* from individual water bottles attached to the cages. Chlorine was added to the filtered water as a bacteriostat. |
| **Environmental conditions:** | **Temperature:** 64-79°F<br>**Humidity:** 30-70%<br>**Air changes:** 10/hour<br>**Photoperiod:** 12 hrs light/dark |
| **Acclimation period:** | At least 5-6 days |

## B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: January 30, 2006; End: February 14, 2006

2. **Study design:** Table 1 shows the treatment groups allocated for the study.

**TABLE 1. Study design for cholinesterase inhibition studies**

| Test Material | Dose(s) (mg/kg/day) | Sex/ No. of animals/ sex/ dose | Treatment and termination |
|---|---|---|---|
| Malathion | 0, 5, 25, 50, 150 | M/F: 12 | Once daily oral dose (PNDs 11-21) to pups; All dams sacrificed on day 21 *postpartum and discarded*; Clinical observations occurred once daily starting day after arrival, during the pre-dose period, prior to dose administration, and 60 ± 10 minutes after dose; clinical observations were performed just prior to sacrifice. Whole blood samples collected at 2 hrs post-dose (malathion) and 30 min post-dose for the pups (malaoxon); *Blood samples stored on cold packs* and brains stored on ice for RBC and Brain cholinesterase assays; Cholinesterase assays conducted on the day of pup sacrifice |
| Malaoxon | 0, 0.1, 1, 2.5, 4 | | |

PND = postnatal day.

3. **Mating procedure:** The female rats were bred at Charles Rivers Labs facility with breeder male rats of the same source and strain. The day of pup birth was designated Day 0 of lactation (postpartum). The dams delivered at the lab facility and were shipped to registrant testing facility on PNDs 4, 5, 7, and 8.

**Animal assignment:** Female rats were assigned temporary animal numbers at arrival. On PNDs 9 or 10, pups selected for study were individually identified by tattoo markings. On PNDs 9 or 10, twenty-four litters of approximately 10 pups/litter (5M/5F) were randomly assigned. The pups from 12 of these litters were assigned to the Malathion dose groups, and the other 12 litters were assigned to the Malaoxon dose groups. One male and one female pup from each of the litters were assigned to each of five respective dose groups. The pups had no adverse clinical signs following physical examination.

5. **Dose selection and time-of-peak effect rationale**: Doses for the pups were selected based on data from a repeat dose range-finding study of Malathion and Malaoxon (TQC00011, not reviewed here) conducted at Charles River Laboratories Preclinical Services (PA). The doses used during the conduct of the study were 5, 15, and 50 mg/kg/day for malathion and 0.05, 0.1, and 1 mg/kg/day for malaoxon. There were no test substance-related adverse clinical effects. Also, minimal cholinesterase inhibition was observed during the study. Preliminary information regarding time-to-peak effect was reviewed by EPA. EPA determined that the times for Malathion (2 hr) and Malaoxon (30 min) were acceptable (see Appendix 1, attached e-mail).

6. **Dose administration:** The pups were administered the test substance and/or the vehicle once daily by gavage from PNDs 11 through 21. Doses were adjusted daily for body weights recorded prior to administration. Dose administration occurred at approximately the same time each day. The dose volume was 5 ml/kg for both test substances and the vehicle.

7. **Dose preparation and analysis:** Prepared suspensions were refrigerated (2°C to 8°C) and protected from light. The test substances were considered 100% pure for the purposes of dose calculations. There were no potential contaminants likely to have been present in the vehicle that would have interfered with the results of the study.

7.1 **Concentration**
Malathion samples prepared on January 30, 2006 was within acceptable limits of ±15% error. Malaoxon samples prepared on February 2, 2006 were within acceptable limits of ±15% error.

7.2 **Homogeneity**
Homogeneity was determined for all dose concentration levels. Mean concentration results from samples taken from the top, middle and bottom of the formulations were calculated. Homogeneity was calculated by determining the percent relative standard deviation (RSD) of the three mean values. All of the results were within the acceptable range of ≤ 5% RSD. Malathion values were 1.0%, 1.5%, 1.0% and 1.3% RSD for the 1, 5, 10, and 30 mg/ml formulations, respectively. Malaoxon values were 1.1%, 2.0%, 0.9% and 0.4% RSD for the 0.02, 0.2, 0.5, and 0.8 mg/ml formulations, respectively.

B. OBSERVATIONS:

1. **In-life observations:**
**PND 11 and young adult animals:** *(Dams)* Rats were observed at least twice daily for mortality. Maternal behavior, clinical observations, and body weights were recorded the day after arrival. Feed consumption was monitored as feed was replenished on an as-needed basis.

*(F1 Generation Pups)* Litters were observed at least twice daily for mortality. Clinical observations were recorded as follows: once daily during the pre-dose period by litter, prior to dose administration, and 60 ± 10 minutes after dose administration. On the last day of the study, clinical observations were performed just prior to sacrifice. Body weights were recorded the day after arrival, on the day of randomization, and daily during the dosing period.

2. **Termination schedule, sample collection, and necropsy procedures** On day 21 postpartum, all dams were sacrificed by carbon dioxide asphyxiation and discarded. All surviving pups were sacrificed by decapitation without anesthesia on PND 21. Sacrifice was immediately followed by blood collection and brain dissection. Pups that died were necropsied for the cause of death. The lungs, trachea, and esophagus were perfused in neutral buffered 10% formalin for possible evaluation.

3. **Cholinesterase determination:** At the end of the dose period (PND 21), blood samples were collected from each of the pups assigned for cholinesterase assay following decapitation. The blood samples were collected at 2 hours post-dose from the M/F pups assigned to the malathion dose groups and 30 minutes post-dose for the pups assigned to the malaoxon dose groups.

Blood samples were stored on cold packs until being processed for RBC cholinesterase levels. The brain was excised and the weight was recorded. The brains were stored on ice until assayed for cholinesterase levels. Cholinesterase assays were conducted on the day of pup sacrifice. The specific analysis method used was not provided.

C. DATA ANALYSIS: Cholinesterase values for red blood cells and brains were evaluated as separate dependent variables in one-way analyses of variance (ANOVA) at each combination of sex (M/F). Sample collection intervals were used as the independent variable in the ANOVA. In the event that the ANOVA was significant ($p \geq 0.05$), the interval with the largest value was compared with the values at each of the other intervals using Dunnett's test.

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## II. RESULTS:

*(Malathion)*

**MORTALITY AND CLINICAL OBSERVATIONS:** One pup in the 5 mg/kg/day dose group was found dead on PND 19, nine days after the initiation of dose administration. Prior to death, the pup had shown signs of weight gain. The carcass was partially cannibalized, but the remaining tissues appeared normal at necropsy. No other deaths occurred. All other pups survived to sacrifice.

Clinical signs related to administration of the test substance were observed in both the male and the female pups in the 150 mg/kg/day dose group during PNDs 11-14. The clinical signs included: tremors in the head or whole body (M/F), decreased motor activity, impaired righting reflex and splayed forelimbs (M), and pale extremities (F). The remaining clinical observation (absent left eye) in one of the 25 mg/kg/day female pups was considered unrelated to the test substance.

**BODY WEIGHT:** There were no significant differences among the dose groups in body weights or body weight changes.

**BRAIN WEIGHT:** Brain weights were not significantly different in any of the malathion dose groups (M/F).

**CHOLINESTERASE ACTIVITY:** M/F pups administered malathion at doses of 25 mg/kg/day and higher had statistically significant reduction ($p \leq 0.05$ to $p \leq 0.01$) in RBC cholinesterase levels as compared to control (Table 1). The levels in the 5 mg/kg/day dose groups were comparable to control. M/F pups administered the 150 mg/kg/day dose of malathion had statistically significant reduction ($p \leq 0.01$) in brain cholinesterase levels as compared with control (Table 2). The values in the 50 mg/kg/day dose and lower were comparable to control.

**Table 1: Malathion RBC Cholinesterase Levels** (1)

| Group | Dosage (mg/kg/day) | Mean ChE ChE U/ml ± S.D. (n) | Percent Decrease Compared with Controls |
|---|---|---|---|
| **Male Pups** | | | |
| I | 0 (Vehicle) | 2.316 ± 0.373 (12) | -- |
| II | 5 | 2.067 ± 0.384 (12) | 10.8 % |
| III | 25 | 1.967 ± 0.383 (12)* | 15.1 % |
| IV | 50 | 1.527 ± 0.276 (12)** | 34.1 % |
| V | 150 | 1.063 ± 0.274 (11)** | 54.1 % |

| Female Pups | | | |
|---|---|---|---|
| **Group** | **Dosage (mg/kg/day)** | **Mean ChE ChE U/ml ± S.D. (n)** | **Percent Decrease Compared with Controls** |
| I | 0 (Vehicle) | 2.119 ± 0.272 (12) | -- |
| II | 5 | 2.022 ± 0.338 (11) | 4.6 % |
| III | 25 | 1.746 ± 0.213 (12)** | 17.6 % |
| IV | 50 | 1.482 ± 0.192 (12)** | 30.1 % |
| V | 150 | 1.024 ± 0.163 (12)** | 51.7 % |

(1) Taken from Text Table 1, p.28, MRID 46822201.
*Significantly different from the vehicle control group value ($p \leq 0.05$).
**Significantly different from the vehicle control group value ($p \leq 0.01$).

| **Table 2: Malathion Brain Cholinesterase Levels**[1] | | | |
|---|---|---|---|
| **Group** | **Dosage (mg/kg/day)** | **Mean ChE CuE U/G ± S.D. (n)** | **Percent Decrease Compared with Controls** |
| **Male Pups** | | | |
| I | 0 (Vehicle) | 12.104 ± 1.706 (10) | -- |
| II | 5 | 12.063 ± 1.038 (12) | 0.3 % |
| III | 25 | 12.292 ± 0.725 (12) | a |
| IV | 50 | 12.093 ± 1.070 (12) | 0.1 % |
| V | 150 | 10.354 ± 1.605 (12)** | 14.5 % |

| Female Pups | | | |
|---|---|---|---|
| **Group** | **Dosage (mg/kg/day)** | **Mean ChE CuE U/G ± S.D. (n)** | **Percent Decrease Compared with Controls** |
| I | 0 (Vehicle) | 11.880 ± 1.791 (12) | -- |
| II | 5 | 12.448 ± 0.856 (11) | b |
| III | 25 | 11.745 ± 0.702 (12) | 1.1 % |
| IV | 50 | 12.170 ± 0.884 (12) | c |
| V | 150 | 9.886 ± 1.643 (12)** | 16.8 % |

(1) Taken from Text Table 2, p.29, MRID 46822201.
**Significantly different from the vehicle control group value ($p \leq 0.01$).
a. No inhibition occurred; value was 1.6% greater than the control value.
b. No inhibition occurred; value was 4.8% greater than the control value
c. No inhibition occurred; value was 2.4% greater than the control value.

***(Malaoxon)***

**MORTALITY AND CLINICAL OBSERVATIONS:** All pups survived until scheduled sacrifice with the exception of one male pup at 1 mg/kg/day found dead on PND 11 and one female pup at 2.5 mg/kg/day found dead on PND 16. These deaths were not considered to be test substance-related because at necropsy it became apparent that they were the result of gavage errors. All other M/F pups survived to scheduled sacrifice.

All clinical observations were not related to the administration of the test substance because: 1) the incidences were not dose dependent; or 2) the observation occurred in only one pup in the dose groups. These clinical observations included a scab on the neck and urine stained abdominal fur.

**BODY WEIGHT:** There were no significant differences among the dose groups in body weights or body weight changes.

**BRAIN WEIGHT:** Brain weights were not significantly different in any of the malaoxon dose groups (M/F).

**CHOLINESTERASE ACTIVITY:** RBC cholinesterase levels were statistically significantly reduced ($p<0.05$ to $p<0.01$) in both the male and female pups administered malaoxon at doses of 1 mg/kg/day and higher as compared to control (Table 3). The values in the 0.1 mg/kg/day dose groups were comparable to control. The brain cholinesterase levels were comparable to control for M/F pups up to 4 mg/kg/day (Table 4).

**Table 3: Malaoxon RBC Cholinesterase Levels**(1)

| Group | Dosage (mg/kg/day) | Mean ChE ChE U/ml ± S.D. (n) | Percent Decrease Compared with Controls |
|---|---|---|---|
| **Male Pups** | | | |
| I | 0 (Vehicle) | 1.930 ± 0.285 (12) | -- |
| VI | 0.1 | 1.895 ± 0.331 (12) | 1.8 % |
| VII | 1 | 1.657 ± 0.188 (10)* | 14.1 % |
| VIII | 2.5 | 1.047 ± 0. 109 (12)** | 45.8 % |
| IX | 4 | 0.943 ± 0.275 (12)** | 51.1 % |
| **Female Pups** | | | |
| I | 0 (Vehicle) | 1.788 ± 0.148 (12) | -- |
| VI | 0.1 | 1.926 ± 0.316 (12) | a |
| VII | 1 | 1.546 ± 0.145 (12)* | 13.5 % |
| VIII | 2.5 | 1.167 ± 0.151 (11)** | 34.7 % |
| IX | 4 | 0.978 ± 0.362 (12)** | 45.3 % |

(1) Taken from Text Table 3, p.31, MRID 46822201.
*Significantly different from the vehicle control group value ($p \leq 0.05$).
**Significantly different from the vehicle control group value ($p \leq 0.01$).
a. No inhibition occurred; value was 7.7% greater than the control value.

**Table 4: Malaoxon Brain Cholinesterase Levels[1]**

| Group | Dosage (mg/kg/day) | Mean ChE ChE U/G ± S.D. (n) | Percent Decrease Compared with Controls |
|---|---|---|---|
| **Male Pups** | | | |
| I | 0 (Vehicle) | 11.867 ± 0.393 (12) | -- |
| VI | 0.1 | 11.897 ± 0.893 (12) | a |
| VII | 1 | 12.131 ± 0.867 (11) | b |
| VIII | 2.5 | 11.746 ± 0.864 (12) | 1.0 % |
| IX | 4 | 11.890 ± 0.857 (12) | c |
| **Female Pups** | | | |
| I | 0 (Vehicle) | 12.311 ± 0.954 (12) | -- |
| VI | 0.1 | 12.065 ± 0.763 (12) | 2.0 % |
| VII | 1 | 12.664 ± 1.939 (12) | d |
| VIII | 2.5 | 12.065 ± 0.938 (11) | 2.0 % |
| IX | 4 | 12.529 ± 0.439 (12) | e |

(1) Taken from Text Table 4, p.32, *MRID* 46822201.

a. No inhibition occurred; value was 0.3% greater than the control value.
b. No inhibition occurred; value was 2.2% greater than the control value.
c. No inhibition occurred; value was 0.2% greater than the control value.
d. No inhibition occurred; value was 2.9% greater than the control value.
e. No inhibition occurred; value was 1.8% greater than the control value.

**AGENCY BMD ANALYSIS:** Benchmark dose analysis was conducted using the OPCumRisk analysis software (http://www.epa.gov/pesticides/cumulative/common_mech_groups.htm#op). Mean cholinesterase levels (Brain, RBC) from the control and dosing groups were placed in a Microsoft Excel file and uploaded into the program. A model run was executed, with the results displayed in Microsoft Explorer files (Table 5, Appendix II).

**Table 5: Benchmark Dosing Analysis of Brain and RBC cholinesterase in rat pups**

| Repeated Only | Compartment | Sex | Subpopulation | $BMD_{10}$ (mg/kg) | $BMDL_{10}$ (mg/kg) | GoF p-value |
|---|---|---|---|---|---|---|
| Malathion | Brain | Male | Adult | 96.67 | 69.43 | 0.474 |
| | | Female | Adult | 76.76 | 58.15 | 0.177 |
| | RBC | Male | Adult | 12.84 | 8.932 | 0.345 |
| | | Female | Adult | 13.12 | 9.955 | 0.994 |
| **Repeated Only** | **Compartment** | **Sex** | **Subpopulation** | **$BMD_{10}$ (mg/kg)** | **$BMDL_{10}$ (mg/kg)** | **GoF p-value** |
| Malaoxon[1] | RBC | Male | Adult | 0.3929 | 0.2842 | 0.0907 |
| | | Female | Adult | 0.4982 | 0.335 | 0.475 |

(1) Brain cholinesterase was not calculated due to a repetitive program/data error in OP Cum Risk software.

## III. DISCUSSION AND CONCLUSIONS:

**INVESTIGATORS' CONCLUSIONS:** "Repeated oral (gavage) administration of malathion to male and female pups during PNDs 11 to 21 resulted in clinical signs (tremors, decreased motor activity, impaired righting reflex, splayed forelimbs and pale extremities) in the 150 mg/kg/day dose group for male and/or female pups during the first few days of administration. There was also a treatment-related statistically significant reduction in RBC cholinesterase activity observed in the 25, 50, and 150 mg/kg/day dose groups for both male and female pups compared with control. Brain cholinesterase activity was also statistically significantly reduced in the 150 mg/kg/day dose group male and female pups compared with control. The NOAEL for cholinesterase inhibition was considered to be 5 mg/kg/day for both the male and female pups administered malathion."

"Repeated oral (gavage) administration of malaoxon to male and female pups during PNDs 11 to 21 resulted in no treatment-related effects other then a statistically significant decrease in RBC cholinesterase levels in the 1, 2.5, and 4 mg/kg/day dosage group male and female pups compared with controls. The NOAEL for cholinesterase inhibition was considered to be 0.1 mg/kg/day for both male and female pups administered maloxon."

**REVIEWER'S COMMENTS:** (Malathion) There were no treatment-related effects on body weight and brain weight. Clinical signs (tremors, decreased motor activity, impaired righting reflex, splayed forelimbs and pale extremities) were observed in the 150 mg/kg/day dose group for M/F pups. Cholinesterase levels were significantly reduced in RBC cholinesterase activity at 25, 50 and 150 mg/kg/day. Brain cholinesterase activity was also statistically significantly reduced in the 150 mg/kg/day M/F pups.

(Malaoxon) There were no treatment-related effects other than a statistically significant decrease in RBC cholinesterase levels in the 1, 2.5, and 4 mg/kg/day M/F pups compared with control.

**Based on a BMD analysis of the RBC cholinesterase data, the point of departure is 9 mg/kg/day ($BMDL_{10}$) for Malathion and 0.3 mg/kg/day ($BMDL_{10}$) for Malaoxon.**

STUDY DEFICIENCIES:

1. Insufficient information was provided about the procedures for the clinical observations. More complete descriptions of these procedures should be provided.

2. The specific cholinesterase assay analytical method need to be provided.

**Appendix I**

Thomas Moriarty/DC/USEPA/US
02/02/2006 04:16 PM To
PW.US@cheminova.com
cc
Louis Scarano/DC/USEPA/US@EPA, Anna Lowit/DC/USEPA/US@EPA, Ginger Moser/RTP/USEPA/US@EPA, Stephanie Padilla/RTP/USEPA/US@EPA, Kathleen Raffaele/DC/USEPA/US@EPA
bcc

Subject
Time to peak for multi-dose comparative ChE with malathion-malaoxon

Dear Paul:

Based upon the data submitted by Cheminova in your email dated 1/30/06, the Agency believes that the time to peak effect for malaoxon should be early, at 30 minutes. This may maximize the brain inhibition and have little effect on the RBC inhibition.

With respect to the time to peak effect for malathion, either 1 hour or 2 hours appears to be acceptable.

If you have any questions, please do not hesitate to contact me.

Regards,
Tom Moriarty
OPP/SRRD
703.305.5035

## Appendix II

### *MALATHION*

```
                    MALATHION:5-D:BRAIN:M:MAIN
                   Wed Feb 28 20:58:53 2007
                  MRID: 1   Guideline: NONE
          Continuous Exponential Model (Decreasing)
          Formula: chei = B + (A-B)*exp(-(m*dose)^g)

Variance Function: power

   The BMD corresponds to a dose that results in a 10% reduction in the
                    response relative to the control
   ------------------------------------------------------------
                    Summary of Model Fitting Results
      AIC       BIC    logLik
205.51142 211.79446 -99.75571
Coefficients:
         Value    Std.Error
A 12.365562173 0.2295999811
m  0.001089940 0.0002600036

Correlation:
          A         m
A 1.0000000 0.6414137
m 0.6414137 1.0000000

Approximate 95% confidence intervals

 Coefficients:
        lower          est.        upper
A 1.191440e+01 12.365562173 12.833804209
m 6.761203e-04  0.001089940  0.001757038

 Residual standard error:
   lower     est.     upper
1.148055 1.356183 1.657193

Degrees of freedom: 60 total; 58 residual
   ------------------------------------------------------------
                          Goodness of Fit
 The chi-squared goodness-of-fit values should be taken as general
indications of fit only.  P-values are likely to be inaccurate to some
degree

Pearson Chi-Square Statistic: 2.507 with 3 degrees of freedom.  P =
0.474

  dose  n   chei Expected    sd   Exp.SD  X2 Resid.
1    0 12 12.104 12.36556 1.706 1.362412 -0.6650542
2    5 12 12.063 12.29836 1.038 1.355227 -0.6015968
3   25 12 12.292 12.03317 0.725 1.326862  0.6757439
4   50 12 12.093 11.70971 1.070 1.292240  1.0274850
5  150 12 10.354 10.50052 1.605 1.162552 -0.4365790
   ------------------------------------------------------------
                          BMD Computation
                    BMD = 96.67:  BMDL = 69.43
   ------------------------------------------------------------
                          Potency Measures
A unit dose (1 mg/kg) would result in 100*exp(-Potency)% of background activity

Potency: 0.00109
se: 0.00026
var=se^2: 6.76e-08
Per cent. of background at unit dose: 100
Per cent. of background at the highest dose: 85
ED50 (95% CI): 635.9 ( 398.4 , 1015 )

ln(Potency) -6.812
se[log(Potency)]: 0.2385
se[log(Potency)]^2: 0.05691
```

MALATHION:5-D:**BRAIN:F**:MAIN
Wed Feb 28 20:58:42 2007
MRID: 1 Guideline: NONE
Continuous Exponential Model (Decreasing)
Formula: chei = B + (A-B)*exp(-(m*dose)^g)

Variance Function: power

The BMD corresponds to a dose that results in a 10% reduction in the response relative to the control

---

Summary of Model Fitting Results

| AIC | BIC | logLik |
|---|---|---|
| 206.8659 | 213.1490 | -100.4330 |

Coefficients:

| | Value | Std.Error |
|---|---|---|
| A | 12.349929363 | 0.2351627259 |
| m | 0.001372676 | 0.0002669472 |

Correlation:

| | A | m |
|---|---|---|
| A | 1.0000000 | 0.6410911 |
| m | 0.6410911 | 1.0000000 |

Approximate 95% confidence intervals

Coefficients:

| | lower | est. | upper |
|---|---|---|---|
| A | 1.188806e+01 | 12.349929363 | 12.829744928 |
| m | 9.300504e-04 | 0.001372676 | 0.002025953 |

Residual standard error:

| lower | est | upper |
|---|---|---|
| 1.192632 | 1.408842 | 1.721539 |

Degrees of freedom: 60 total; 58 residual

---

Goodness of Fit

The chi-squared goodness-of-fit values should be taken as general indications of fit only. P-values are likely to be inaccurate to some degree

Pearson Chi-Square Statistic: 4.933 with 3 degrees of freedom. P = 0.177

| | dose | n | chei | Expected | sd | Exp.SD | X2 Resid. |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 12 | 11.880 | 12.34993 | 1.791 | 1.395105 | -1.1668534 |
| 2 | 5 | 12 | 12.448 | 12.26546 | 0.856 | 1.385874 | 0.4562797 |
| 3 | 25 | 12 | 11.745 | 11.93331 | 0.702 | 1.349558 | -0.4833561 |
| 4 | 50 | 12 | 12.170 | 11.53074 | 0.884 | 1.305498 | 1.6962571 |
| 5 | 150 | 12 | 9.886 | 10.05177 | 1.643 | 1.143179 | -0.5023303 |

---

BMD Computation

**BMD = 76.76: BMDL = 58.15**

---

Potency Measures

A unit dose (1 mg/kg) would result in 100*exp(-Potency)% of background activity

Potency: 0.001371
se: 0.0002669
var=se^2: 7.125e-08
Per cent of background at unit dose: 100
Per cent of background at the highest dose: 81
ED50 (95% CI): 505 ( 344.9 , 739.3 )

ln(Potency) -6.591
se[log(Potency)]: 0.1945
se[log(Potency)]^2: 0.03782

MALATHION:5-D:**RBC**:**M**:MAIN
Wed Feb 28 20:59:02 2007
MRID: 1 Guideline: NONE
Continuous Exponential Model (Decreasing)
Formula: chei = B + (A-B)*exp(-(m*dose)^g)

Variance Function: power

The BMD corresponds to a dose that results in a 10% reduction in the response relative to the control

Summary of Model Fitting Results

| AIC | BIC | logLik |
|---|---|---|
| 47.18893 | 55.56631 | -19.59447 |

Coefficients:

| | Value | Std.Error |
|---|---|---|
| A | 2.26873075 | 0.097573224 |
| B | 0.89262663 | 0.176193868 |
| m | 0.01403654 | 0.005162518 |

Correlation:

| | A | B | m |
|---|---|---|---|
| A | 1.0000000 | 0.4381392 | 0.6015751 |
| B | 0.4381392 | 1.0000000 | 0.9211930 |
| m | 0.6015751 | 0.9211930 | 1.0000000 |

Approximate 95% confidence intervals

Coefficients:

| | lower | est. | upper |
|---|---|---|---|
| A | 2.081520851 | 2.26873075 | 2.47277811 |
| B | 0.601186710 | 0.89262663 | 1.32534917 |
| m | 0.006720582 | 0.01403654 | 0.02931659 |

Residual standard error:

| lower | est. | upper |
|---|---|---|
| 0.3902883 | 0.4616576 | 0.5652132 |

Degrees of freedom: 60 total; 57 residual

Goodness of Fit

The chi-squared goodness-of-fit values should be taken as general indications of fit only. P-values are likely to be inaccurate to some degree

Pearson Chi-Square Statistic: 2.127 with 2 degrees of freedom. P = 0.345

| | dose | n | chei | Expected | sd | Exp.SD | X2 Resid. |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 12 | 2.316 | 2.268731 | 0.373 | 0.4508786 | 0.36316979 |
| 2 | 5 | 12 | 2.067 | 2.175463 | 0.384 | 0.4329388 | -0.86785359 |
| 3 | 25 | 12 | 1.967 | 1.861465 | 0.383 | 0.3720423 | 0.98263774 |
| 4 | 50 | 12 | 1.527 | 1.574732 | 0.276 | 0.3155828 | -0.52394986 |
| 5 | 150 | 12 | 1.063 | 1.060218 | 0.274 | 0.2106011 | 0.04575537 |

BMD Computation

**BMD = 12.84: BMDL = 8.932**

Potency Measures

A unit dose (1 mg/kg) would result in 100*exp(-Potency)% of background activity

Potency: 0.01404
se: 0.005163
var=se^2: 2.665e-05
Per cent. of background at unit dose: 99
Per cent. of background at the highest dose: 12
ED50 (95% CI): 49.38 ( 24.02 , 101.5 )

ln(Potency) -4.266
se[log(Potency)]: 0.3678
se[log(Potency)]^2: 0.1353

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```
                    MALATHION:5-D:RBC:F:MAIN
                    Wed Feb 28 20:58:51 2007
                    MRID: 1   Guideline: NONE
          Continuous Exponential Model (Decreasing)
          Formula: chei = B + (A-B)*exp(-(m*dose)^g)

Variance Function: power

   The BMD corresponds to a dose that results in a 10% reduction in the
                        response relative to the control
    ------------------------------------------------------------------
                        Summary of Model Fitting Results

      AIC       BIC    logLik
-2.549624  5.827754  5.274812

Coefficients:
      Value   Std.Error
A 2.1142937 0.064052979
B 0.8800655 0.113075518
m 0.0143269 0.003781777

Correlation:
          A         B         m
A 1.0000000 0.4251433 0.5976098
B 0.4251433 1.0000000 0.9136258
m 0.5976098 0.9136258 1.0000000

Approximate 95% confidence intervals

 Coefficients:
       lower      est.      upper
A 1.989842940 2.1142937 2.24652807
B 0.680419044 0.8800655 1.13829156
m 0.008444888 0.0143269 0.02430583

 Residual standard error:
    lower      est.     upper
0.2505508 0.2963563 0.3628461

Degrees of freedom: 60 total; 57 residual
    ------------------------------------------------------------------
                              Goodness of Fit
 The chi-squared goodness-of-fit values should be taken as general
indications of fit only.  P-values are likely to be inaccurate to some
degree

Pearson Chi-Square Statistic: 0.01274 with 2 degrees of freedom.  P =
0.994

  dose  n  chei Expected    sd    Exp.SD      X2 Resid.
1    0 12 2.119 2.114294 0.272 0.2957086  0.0551318356
2    5 12 2.022 2.028973 0.338 0.2837755 -0.0851190623
3   25 12 1.746 1.742732 0.213 0.2437414  0.0464395280
4   50 12 1.482 1.483029 0.192 0.2074188 -0.0171803036
5  150 12 1.024 1.023972 0.163 0.1432145  0.0006690805
    ------------------------------------------------------------------
                              BMD Computation
                        BMD = 13.12:  BMDL = 9.955
    ------------------------------------------------------------------
                              Potency Measures
A unit dose (1 mg/kg) would result in 100*exp(-Potency)% of background activity
Potency: 0.01433
se: 0.003782
var=se^2: 1.43e-05
Per cent. of background at unit dose: 99
Per cent. of background at the highest dose: 12
ED50 (95% CI): 48.38 ( 28.84 , 81.16 )

ln(Potency) -4.246
se[log(Potency)]: 0.264
se[log(Potency)]^2: 0.06968
```

## *MALAOXON*

MALAOXON:5-D:**RBC:M**:MAIN
Wed Feb 28 20:58:40 2007
MRID: 2 Guideline: NONE
Continuous Exponential Model (Decreasing)
Formula: chei = B + (A-B)*exp(-(m*dose)^g)

Variance Function: power

The BMD corresponds to a dose that results in a 10% reduction in the response relative to the control

Summary of Model Fitting Results

| AIC | BIC | logLik |
|---|---|---|
| 16.54032 | 24.91770 | -4.27016 |

Coefficients:

| | Value | Std.Error |
|---|---|---|
| A | 1.9749443 | 0.0768238 |
| B | 0.6752369 | 0.2280053 |
| m | 0.4194937 | 0.1673847 |

Correlation:

| | A | B | m |
|---|---|---|---|
| A | 1.0000000 | 0.3711758 | 0.4889201 |
| B | 0.3711758 | 1.0000000 | 0.9687245 |
| m | 0.4889201 | 0.9687245 | 1.0000000 |

Approximate 95% confidence intervals

Coefficients:

| | lower | est. | upper |
|---|---|---|---|
| A | 1.8269462 | 1.9749443 | 2.1349314 |
| B | 0.3434004 | 0.6752369 | 1.3277357 |
| m | 0.1886763 | 0.4194937 | 0.9326819 |

Residual standard error:

| lower | est. | upper |
|---|---|---|
| 0.3036824 | 0.3592142 | 0.4397911 |

Degrees of freedom: 60 total; 57 residual

*Goodness of Fit*

The chi-squared goodness-of-fit values should be taken as general indications of fit only. P-values are likely to be inaccurate to some degree

Pearson Chi-Square Statistic: 4.799 with 2 degrees of freedom. P = 0.0907

| | dose | n | chei | Expected | sd | Exp.SD | X2 Resid. |
|---|---|---|---|---|---|---|---|
| 1 | 0.0 | 12 | 1.930 | 1.9749443 | 0.285 | 0.3672401 | -0.4239502 |
| 2 | 0.1 | 12 | 1.895 | 1.9215501 | 0.331 | 0.3573232 | -0.2573925 |
| 3 | 1.0 | 12 | 1.657 | 1.5296380 | 0.188 | 0.2845647 | 1.5504208 |
| 4 | 2.5 | 12 | 1.047 | 1.1306296 | 0.109 | 0.2105683 | -1.3758078 |
| 5 | 4.0 | 12 | 0.943 | 0.9179596 | 0.275 | 0.1711836 | 0.5067220 |

BMD Computation

**BMD = 0.3929: BMDL = 0.2842**

Potency Measures

A unit dose (1 mg/kg) would result in 100*exp(-Potency)% of background activity

Potency: 0.4195
se: 0.1674
var=se^2: 0.02802
Per cent. of background at unit dose: 66
Per cent. of background at the highest dose: 19
ED50 (95% CI): 1.652 ( 0.7559 , 3.612 )

ln(Potency) -0.8687
se[log(Potency)]: 0.399
se[log(Potency)]^2: 0.1592

```
                    MALAOXON:5-D:RBC:F:MAIN
                  Wed Feb 28 20:58:28 2007
                 MRID: 2   Guideline: NONE
          Continuous Exponential Model (Decreasing)
          Formula: chei = B + (A-B)*exp(-(m*dose)^g)

Variance Function: power

   The BMD corresponds to a dose that results in a 10% reduction in the
                    response relative to the control
    ------------ ------------------------------------------------------
                    Summary of Model Fitting Results

      AIC       BIC    logLik
25.166264 33.542643 -8.583132

Coefficients:
      Value  Std.Error
A 1.8790348 0.07800574
B 0.6459034 0.39183037
m 0.3318426 0.20351731

Correlation:
          A         B         m
A 1.0000000 0.3944732 0.4896534
B 0.3944732 1.0000000 0.9819996
m 0.4896534 0.9819996 1.0000000

Approximate 95% confidence intervals

 Coefficients:
       lower      est.    upper
A 1.72914740 1.8790348 2.041915
B 0.19168925 0.6459034 2.176393
m 0.09717943 0.3318426 1.133156

 Residual standard error:
    lower      est.     upper
0.3249548 0.3843766 0.4705977

Degrees of freedom: 60 total; 57 residual
    ------------ ------------------------------------------------------
                          Goodness of Fit
 The chi-squared goodness-of-fit values should be taken as general
indications of fit only.  P-values are likely to be inaccurate to some
degree

Pearson Chi-Square Statistic: 1.489 with 2 degrees of freedom.  P =
0.475

  dose  n  chei  Expected    sd     Exp.SD  X2 Resid.
1  0.0 12 1.788 1.8790348 0.148 0.3739245 -0.8433626
2  0.1 12 1.926 1.8387858 0.316 0.3663117  0.8247589
3  1.0 12 1.546 1.5307989 0.145 0.3070691  0.1714868
4  2.5 12 1.167 1.1838211 0.151 0.2375415 -0.2453050
5  4.0 12 0.978 0.9728974 0.362 0.1929873  0.0915917
    ------------ ------------------------------------------------------
                          BMD Computation
                    BMD = 0.4982:  BMDL = 0.335
    ------------ ------------------------------------------------------
                          Potency Measures
A unit dose (1 mg/kg) would result in 100*exp(-Potency)% of background activity

Potency: 0.3318
se: 0.2035
var=se^2: 0.04142
Per cent. of background at unit dose: 72
Per cent. of background at the highest dose: 27
ED50 (95% CI): 2.089 ( 0.6278 , 6.949 )

ln(Potency) -1.103
se[log(Potency)]: 0.6133
se[log(Potency)]^2: 0.3761
```

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# R143137

**Chemical:** Malathion

**PC Code:** 057701
**HED File Code:** 13000 Tox Reviews
**Memo Date:** 3/30/2007
**File ID:** TX0054537
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