EPA Reviewer: Susan Makris Signature_____
Toxicology Branch, Health Effects Division (7509C) Date_____

EPA Reviewer: Brian Dementi Signature_____
Toxicology Branch, Health Effects Division (7509C) Date_____

TXR#: 0050550

DATA EVALUATION RECORD

STUDY TYPE: Special Study, Effects on Cholinesterase in Adult and Juvenile CD Rats, Companion Study to Developmental Neurotoxicity Study 870.6300

PC CODE: 057701 **DP BARCODE:** D280071

SUBMISSION NO.: S608281

TEST MATERIAL (PURITY): Malathion (96.0% a.i.)

SYNONYMS: Butanedioic acid, ((dimethoxyphosphinothioyl)thio)-, diethyl ester

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 Unpublished

SPONSOR: Cheminova A/S, P.O. Box 9, DK-7260 Lemvig, Denmark

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.

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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)

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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.

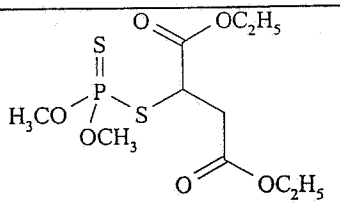
This study is classified acceptable/nonguideline for the determination of plasma, RBC, and brain cholinesterase activities following treatment with malathion in adult, fetal, and juvenile rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and No Data Confidentiality statements were provided.

MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	Malathion
Description:	clear pale pink liquid
Lot/Batch #:	9010501
Purity:	96.0 % a.i.
Compound Stability:	1 year (stored refrigerated during study)
CAS # of TGAI:	121-75-5

Structure:	

2. Vehicle and/or positive control: corn oil was used as the negative control vehicle in this study; no positive control substance was tested

3. Test animals (P):	
Species:	rat
Strain:	Crl:CD® BR
Age and wt. at study initiation:	Virgin females - 10-11 weeks - 214-303 g; Male and females, 7-8 weeks, males 223-319 females 161-231 g
Source:	Charles River UK Ltd., Margate, Kent, England
Housing:	stainless steel cages with grid floors, or polypropylene cages with wood shavings used fo bedding from GD17 to LD14-18
Diet:	Certified UAR VRF1 pelleted rodent diet, Charles River UK Ltd., *ad libitum*
Water:	tap water, *ad libitum*
Environmental conditions:	**Temperature:** 19-25°C (nominal) **Humidity:** 40-70% (nominal) **Air changes:** Not provided **Photoperiod:** 12 hrs light/dark
Acclimation period:	Virgin females - 9-10 weeks - at least 5 days; Males and females - 5-6 weeks - at least 12 days

B. PROCEDURES AND STUDY DESIGN

1. **In life dates** - Start: November 2, 2000 End: January 25, 2001

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

Table 1. Study Design

Group	Malathion Dose (mg/kg/day)	Number of animals/sex	Treatment
1	0	19 F	Nine treated from GD 6 to GD 20; ten treated from GD 6 to PND 10 with offspring from 8 litters treated from PND 11 through PND 21
2	5	19 F	Nine treated from GD 6 to GD 20; ten treated from GD 6 to PND 10 with offspring from 8 litters treated from PND 11 through PND 21
3	50	19 F	Nine treated from GD 6 to GD 20; ten treated from GD 6 to PND 10 with offspring from 8 of the litters treated from PND 11 through PND 21
4	150	19 F	Nine treated from GD 6 to GD 20; ten treated from GD 6 to PND 10 with offspring from 8 litters treated from PND 11 through PND 21
5	0	8 F	No treatment of dams. On PND 11, one male and one female offspring/litter were treated with 0, 5, 50, 150, or 450 mg/kg malathion.
6	0	16 F/16 M	Eight males and females were treated for one day; eight males and females were treated for 11 consecutive days.
7	5	16 F/16 M	Eight male and females were treated for one day; eight male and females treated for 11 consecutive days.
8	50	16 F/16 M	Eight male and females were treated for one day; eight male and females treated for 11 consecutive days.
9	150	16 F/16 M	Eight male and females were treated for one day; eight male and females treated for 11 consecutive days.
10	0	8F/8M	Eight males and females were treated for one day. a

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Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for spermatozoa. The day a vaginal smear tested positive for sperm or at least three copulation plugs were found was designated GD 0.

4. **Animal Assignment:** Mated female rats in Groups 1-4 (Table 1) showing unequivocal evidence of mating were allocated to group and cage positions in sequence to attempt to ensure that animals mated on any one day were evenly distributed among groups.

   Young adult male and female rats in Groups 6-9 were allocated based on sex; litter mates (information provided by supplier) were not assigned to the same group.

   Young adult male and female rats in Groups 10-11, which were received and placed on study after dosing of Groups 6-9 has been completed, were allocated based on sex and weight (5 g blocks). Rats were randomly selected from each block by rotating to compose the treatment groups.

   Offspring from mated female rats in Group 5 were assigned to one of the four treatment groups as follows: one male and one female pup from each litter with the lowest within-litter identity numbers for each sex were assigned to the control group; one male and one female pup with the second lowest identity number were treated with 5 mg/kg test material; one male and one female pup with the third lowest identity number were treated with 50 mg/kg test material; one male and one female pup with the second highest identity number were treated with 150 mg/kg test material and one male and one female pup with the highest identity number were treated with 450 mg/kg test material.

5. **Dose selection rationale:** Dose levels were selected by the Sponsor based on a dose-finding study in CD rats (MRID 45627001). In that study, it was concluded that 150 mg/kg/day would be suitable for use as the highest dosage on a developmental neurotoxicity study. The report states (p. 19) that "Dosages higher than 150 mg/kg/day were considered likely to lead to adverse consequences in directly dosed offspring which would have the potential to compromise assessment of aspects of neurotoxicity in the offspring, other than reduction in cholinesterase activity." A complete evaluation of treatment-related effects on adults and offspring that were observed in the dose-finding study can be found in TXR 0050550. Signs of toxicity in offspring included whole body tremors and/or morbidity following only the second or third day of gavage dosing (initiated on PND 11) at 200 mg/kg/day; severe clinical observations and mortality were reported at doses of 450 mg/kg/day and above.

   After the study had commenced, an additional dose level of 450 mg/kg/day was added for the evaluation of cholinesterase inhibition in PND 11 (Group 5) and young adult rats (Group 11) following acute doses.

6. **Dosage administration:** All single or multiple doses were administered to the adult males and females, mated dams, and selected offspring in the groups shown in Table 1 by daily oral gavage at a volume of 5 mL/kg/day calculated from the most recent body weight.

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7. **Dosage preparation and analysis:** Formulations were prepared weekly. Each dose concentration (1, 10, 30, and 90 mg/ml) was prepared by adding the required amount of test

substance to an appropriate amount of corn oil and homogenizing using a high shear homogenizer. Prior to the start of the study, homogeneity and stability of the test substance was evaluated as part of the developmental neurotoxicity study (MRID 45646401). Samples were obtained for determination of test material concentration from solutions prepared for use during the first week of dosing. The 90 mg/ml dose formulation was also sampled during the last week of use, however, lower concentration formulations were erroneously not sampled for concentration analysis.

Results - Homogeneity and Stability Analyses: Trial formulations of 1 and 250 mg/ml malathion in corn oil were prepared prior to initiation of treatment. These samples were evaluated for homogeneity and re-suspendibility. The results (MRID 45656501, Addendum 7, page 1352) demonstrated that a suspension of malathion (formulated in corn oil) was homogeneous. This suspension was maintained for up to 2 hours (while on a magnetic stirrer) and was successfully re-suspended following ambient temperature storage for 2 days and refrigerated storage (4°C) for 15 days.

Concentration Analysis: Mean concentrations of malathion in formulations prepared for the first day of dosing ranged from 91.0 to 101% of nominal; the single analysis performed for the high-dose formulation from the last day of dosing was 109% of nominal. It is also noted that in the definitive developmental neurotoxicity study (MRID 45646401), mean concentrations of malathion in formulations prepared for the first day of dosing ranged from 92.6 to 97.4% of nominal and the mean concentrations from the last day of dosing ranged from 94.9 to 97.0% of nominal (Addendum 7, page 1353). These data, which were all within ±9% of nominal, do not indicate any problem with formulation procedures; therefore, the lack of concentration analyses for the low and mid-dose formulations during the last week of use is not expected to compromise the integrity of the study.

The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. **Adult animals:** All animals were checked at least twice daily for clinical signs or ill health. A full physical examination was conducted daily on each day of the study until parturition, and then weekly during lactation. In addition, detailed observations of all rats were made: prior to treatment, as each animal was returned to the cage, at the end of dosing for each group, between 1 and 2 hours after completion of dosing, and as late as possible during the work day.

Adult males and females in Groups 6-9 were weighed on the day before initial treatment and daily thereafter until study termination. Adult males and females in Groups 10-11 were weighed on the day before treatment and on the day of treatment. Mated females were weighed on GDs 0, 3, 6, 10, 14, 17, 20, and daily thereafter until parturition. During lactation, females were weighed on PNDs 1, 4, 7, 11, 14, 17, and 21.

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b. **Offspring:** The day of completion of parturition was designated as lactation day

(postnatal day) 0. On PND 1, litters were examined for number of live and dead offspring, individual pup body weights, sex ratio, and observations. Offspring were examined daily from PND 1 through 21 for general clinical signs. Dosing observations of all offspring were made on each day of dosing, prior to treatment, as each pup was returned to the home cage, at the end of dosing for each group, between 1 and 2 hours after completion of dosing, and as late as possible during the work day. Selected F1 offspring were subjected to weekly full physical examinations from weaning through study termination.

Daily records were kept on litter mortality and size. On PND 4, litters were standardized to 8 pups/litter (4/sex/litter, when possible) for Groups 1-4 and to 10 pups/litter (5/sex/litter, when possible) for Group 5. The sex of offspring was determined on PND 1, 4, and 21 (Groups 1-4), and PNDs 1, 4, and 11 (Group 5).

Dosed offspring were weighed on PND 1, 4, 7, 11-21, and 28, and then weekly until termination and on PND 60 where appropriate. Group 5 offspring were weighed on PND 1, 4, 7, and 11. Non-dosed offspring were weighed individually prior to weaning on PND 1, 4, 7, 11, 14, 17, and 21.

2. **Termination schedule and sample collection**

Adults and/or offspring were terminated according to the schedule shown in Table 2. No rationale was provided regarding the selection of time of sacrifice in relation to time of dosing. No data were provided to demonstrate the time-to-peak-effect for either adults or offspring.

Table 2. Termination Schedule

Group (s)	Day	Samples	Animals
1 - 4	GD 20	Blood/brain	8 dams/group and fetuses. Dams were killed 3 hours after dosing.
1 - 4	PND 4	Blood/brain	Up to 2 male and 2 female pups in each litter were killed 4 hours after dosing of the dam.
5	PND 11	Blood/brain	All offspring in each litter were killed 2 hours after dosing.
6 - 9	Day 1	Blood/brain	8 males and 8 females/group were killed 2 hours after dosing.
1 - 4	PND 21	Blood/brain	One male and one female offspring in each litter (up to 8 litters/group) were killed 2 hours after dosing
6 - 9	Day 11	Blood/brain	8 males and 8 females/group were killed 2 hours after dosing.
1 - 4	PND 60	Blood/brain	8 males and 8 females/group killed

Data from p 29, MRID 45566201.

Blood samples were collected from the retro-orbital sinus under light isoflurane anesthesia (all adults and PND 21 pups) or from the umbilical cord (GD 20 fetuses). Blood samples from PND 4 and PND 11 pups were collected from the torso following decapitation. All blood samples were collected into tubes containing heparin as an anticoagulant; sample volume was approximately 0.7 ml. Samples were packed on water ice and taken to the Clinical Pathology Department for processing and centrifugation. Resulting samples were stored at -80° C and shipped in dry ice to Huntingdon Research Centre for analysis. Blood samples from fetuses within each litter were pooled prior to analysis; fetal samples were not separated by gender.

With the exception of PND 4 and PND 11 pups which were sacrificed by decapitation, all adults and offspring were sacrificed by CO_2 inhalation. GD 20 fetuses were sacrificed by

chilling on a cool plate. The brains were removed, weighed (fetal brains were pooled by litter), wrapped in aluminum foil and quick frozen in liquid nitrogen. All samples were stored at -80°C until analysis.

3. **Cholinesterase determination**

Cholinesterase assays were performed on all red blood cell (RBC), plasma, and brain samples. The method of analysis was a modified Ellman method. Erythrocyte cholinesterase activity was measured by following the hydrolysis of acetylthiocholine to thiocholine and its subsequent reaction with 6,6'-dithiodinicotinic acid (DTNA) to form a colored product. Plasma and brain cholinesterase activity were measured by following the action of thiocholine on 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to form a colored product. All cholinesterase assays were done on a Hitachi 911 clinical analyzer.

4. **Necropsy procedures**

All animals underwent a detailed macroscopic necropsy. In addition, the reproductive tract of adult GD 20 females, complete with ovaries, was dissected out and the following recorded: number of corpora lutea in each ovary, number of implantation sites, number of resorption sites (classified as early or late), and the number, distribution, and sex of fetuses in each uterine horn. The number of implantation sites was also recorded for dams that had littered and that were killed on either PND 11 (Group 5) or PND 21 (Groups 1-4).

E. DATA ANALYSIS

1. **Statistical analyses:** For parametric data, statistical evaluations were done by ANOVA followed by a test for monotonicity of dose-response (Healey's test). If the dose-response was monotonic, Williams' test was applied; if it was not monotonic, Dunnett's test was performed. Nonparametric data (as evidenced by a significant Bartlett's test at the 1% level, even following logarithmic or square-root transformation of the data), were evaluated by the Kruskal-Wallis test followed by a test for monotonicity of dose response. If the test for monotonicity of dose response was not significant at the 1% level, Shirley's test for monotonic trend was performed. The basic sample unit for litter data was the litter. Where 75% or more of the values for a given variable were the same, Fisher's exact test was used. For all statistical analyses, the level of significance was $p \leq 0.05$.

2. **Indices:**

a. **Reproductive indices:** The following indices were calculated for animals killed on GD 20:

Pre-implantation loss (%) = ([No. corpora lutea-No. implantations]/No. corpora lutea) × 100

Post-implantation loss (%) = ([No. implants - No. live fetuses]/No. implants) × 100

Gestation index = No. of live litters born/number pregnant x 100

b. **Offspring viability indices:** The following viability (survival) indices were calculated from lactation records of litters in the study:

Post-implant. survival index = (Total no. offspring born/Total no. implant. sites) × 100

Live Birth Index = (No. live offspring day 1/Total no. offspring born) × 100

Viability Index = (No. live offspring day 4 precull/No. live offspring day 1) × 100

Lactation Index = (No. live offspring day 7 or 11/No. live offspring day 4 postcull) × 100

II. RESULTS

A. Mortality and clinical and functional observations:

All adult animals survived to individual group termination. Offspring survival was not affected by repeated *in utero* and/or postnatal treatment at maternal dose levels of up to 150 mg/kg/day (Table 3). However, following an acute dose of malathion at 450 mg/kg, one pup was moribund at 1 hour post-dose and was humanely killed. The study report indicates that " isolated mortalities" occurred in pups that were directly dosed from PND 11-21 and were not judged to be associated with treatment; however, these data were not included in the report.

Post-dose salivation was observed during gestation and/or lactation in 3, 4, 5, and 19 dams in the 0, 5, 50, and 150 mg/kg/day dose groups, respectively (Appendix 1, pages 113-120). There were no other treatment-related clinical signs in dams. For young adult animals that received an acute dose of malathion, no clinical observations were noted at any dose, up to and including 450 mg/kg. For adult animals receiving repeated doses of malathion, no treatment-related general clinical signs were observed. Clinical signs were predominantly restricted to staining of the coat and hair loss, and were observed in dams of all control and treated groups.

In PND 11 offspring, body tremors were observed in 5 of 16 neonates at 1-2 hours following an acute dose of 450 mg/kg. The study report states that there were no treatment-related general clinical signs in pups that were administered repeated direct gavage doses of malathion from PND 11-21; however, these data were not included in MRID 45566201.

B. Body weight and food consumption:

Body weight and body weight gain during gestation and lactation were similar between control and treated dams. No treatment-related effects on body weight or body weight gain were observed for adult male or female rats, fetuses, or pups. Fetal and pup weights from treated dams are shown in Table 3.

C. Reproductive performance and litter data: The reproductive performance data for dams that were killed at cesarean section or that were allowed to litter are summarized in Table 3. No differences were observed between the treated and control groups in the mean numbers of corpora lutea, implantations, live fetuses, resorptions, fetal body weights, or fetal sex ratios. In addition, no differences in gestation length, viability, or lactation indices were reported.

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TABLE 3. Reproductive Performance and Offspring Survival from Treated Dams[a]

Observation	Dose (mg/kg/day)			
	0	5	50	150
GD 20 Cesarean Section				
No. Dams (Litters)	9	9	9	9
Corpora Lutea	19.1 ± 4.3	18.6 ± 2.6	16.0 ± 1.5*	16.1 ± 1.3*
Implantations	17.0 ± 1.7	16.9 ± 1.1	16.0 ± 1.4	16.3 ± 1.1
Mean Total Resorptions	0.9	1.1	0.6	0.6
Live Fetuses	16.1 ± 2.1	15.8 ± 2.0	15.4 ± 1.7	15.8 ± 1.6
Mean Pre-implantation Loss (%)	9.2	8.5	1.3	2.0
Mean Post-implantation Loss (%)	5.2	6.7	3.5	3.5
Fetal Weight (g)	4.01 ± 0.17	3.90 ± 0.22	3.92 ± 0.23	3.97 ± 0.27
Natural Delivery				
No. Dams (Litters)	10	10	10	10
Mean Gestation Length (days) b	22.2 ± 0.05	22.1 ± 0.04	22.3 ± 0.26	22.4 ± 0.15
Gestation Index (%)	100	100	100	100
Live Litter size				
Day 1	15.0 ± 1.6	15.7 ± 1.8	13.9 ± 1.6	14.2 ± 1.9
Day 4 (precull)	14.7 ± 1.8	15.0 ± 2.4	13.8 ± 1.5	14.1 ± 2.0
Day 11	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
Pup Deaths				
Birth (Stillborn)	0	0	0	0
Days 1-4	3	7	1	1
Days 5-11	0	0	0	0
Live Birth Index (%)	100	100	100	100
Viability Index (%)	97.9	95.2	99.4	99.2
Lactation Index (Day 11) (%)	100	100	100	100
Post-implantation survival index (%)	92.2	92.3	89.3	94.3
Pup Body Wt. (Male)				
Day 1 c	6.5 ± 0.3	6.1 ± 0.4	6.7 ± 0.7	6.9 ± 0.6
Day 4 (precull) c	9.2 ± 0.7	8.4 ± 0.9	9.7 ± 1.1	9.5 ± 0.8
Day 11 c	27.0 ± 1.9	24.0 ± 2.1**	27.4 ± 1.9	26.1 ± 2.1
Day 14 d	35.4 ± 4.1	32.3 ± 3.8**	35.9 ± 2.7	34.2 ± 2.8
Day 21 d	55.7 ± 6.7	52.5 ± 5.7	56.9 ± 3.7	55.0 ± 5.6
Pup Body Wt. (Female)				
Day 1 c	6.2 ± 0.4	5.9 ± 0.4	6.4 ± 0.6	6.4 ± 0.5
Day 4 (precull) c	8.8 ± 0.8	8.3 ± 1.0	9.2 ± 1.0	9.0 ± 1.0
Day 11 c	25.8 ± 2.3	24.2 ± 2.2	26.4 ± 2.4	25.2 ± 2.1
Day 14 d	34.3 ± 4.6	32.6 ± 3.8	34.5 ± 3.2	32.8 ± 3.4
Day 21 d	54.0 ± 7.3	51.8 ± 5.2	54.6 ± 5.2	52.2 ± 5.7

a Data obtained from pages 70, 71, 72, 74, 75, 76, 77, 79, 82, 84, and 144-147 in MRID 45566201
b Calculated by reviewer.
c Calculated from individual pup weights; includes all pups in litter, prior to direct dosing.
d Calculated from individual pup weights; includes only pups that were directly dosed.
* $p \leq 0.05$, ** $p \leq 0.01$

D. Postmortem Results: No grossly observable treatment-related postmortem abnormalities were observed at necropsy in adult male or female rats, fetuses, or pups.

E. Brain Weights: No treatment-related effects were found on the brain weights of treated dams, adult male and female rats, fetuses, or pups. Offspring brain weights are presented in Table 4.

TABLE 4. Offspring Brain Weight (g) [a]

Observation	Dose (mg/kg/day)				
	0	5	50	150	450

GD 20 Fetuses (n = 8)	0.158 ± 0.02	0.167 ± 0.01	0.154 ± 0.01	0.167 ± 0.01	
PND 4 Male (n= 17, 16, 13, 15) Female (n = 18, 17, 19, 18)	 0.407 ± 0.043 0.400 ± 0.044	 0.396 ± 0.043 0.386 ± 0.038	 0.414 ± 0.060 0.416 ± 0.044	 0.421 ± 0.036 0.414 ± 0.043	
PND 11 (Group 5) (n = 8) Male Female	 1.008 ± 0.051 0.980 ± 0.047	 0.993 ± 0.049 0.984 ± 0.057	 1.019 ± 0.034 0.994 ± 0.084	 1.021 ± 0.059 0.971 ± 0.060	 0.998 ± 0.046 1.004 ± 0.078
PND 21 (n = 8) Male Female	 1.493 ± 0.063 1.471 ± 0.043	 1.457 ± 0.069 1.429 ± 0.077	 1.520 ± 0.080 1.419 ± 0.053	 1.479 ± 0.057 1.428 ± 0.063	
PND 60 (n = 8) Male Female	 2.016 ± 0.090 1.862 ± 0.066	 2.003 ± 0.081 1.852 ± 0.051	 1.990 ± 0.047 1.868 ± 0.108	 1.975 ± 0.062 1.829 ± 0.050	

[a] Data obtained from pages 73, 77, 81, 90, 91, and 98 in MRID 45566201

F. Cholinesterase Activity: The plasma, RBC, and brain cholinesterase activity data for treated adult male and female rats, fetuses, and offspring are shown in Tables 5a, 5b, and 5c. Biologically significant treatment-related effects are bolded.

1. Acute exposures (Table 5a)

In adults, acute exposure to 450 mg/kg malathion resulted in statistically significant inhibition (24%) in plasma cholinesterase in males, and in red blood cell cholinesterase in both sexes (25 and 17%, male and female, respectively). At that dose, brain cholinesterase was not affected in either sex. No effects were seen in any compartment at 150 mg/kg or below.

In PND 11 pups, an acute dose of 5 mg/kg resulted in statistically significant inhibition (16%) in red blood cell cholinesterase in males. At 50 mg/kg, both plasma and red blood cell cholinesterase were significantly inhibited in both male (19% and 25%) and female (16% and 23%) pups. While the 16% inhibition in female pup plasma cholinesterase activity at 50 mg/kg could be considered somewhat marginal it was nevertheless considered treatment-related. Nonsignificant decreases in brain cholinesterase activity were observed in males (6%) and females (10%) at 50 mg/kg. The effect in females, even though not statistically significant, was considered to represent a threshold response to treatment, due to the magnitude of the inhibition and because of the remarkable dose-response observed in the brain cholinesterase data. The magnitude of the response in males was considered more equivocal in interpretation, even though there was a similar dose response as seen in the females. At 150 and 450 mg/kg, plasma, red blood cell, and brain cholinesterase were significantly and substantially inhibited for both sexes. Brain cholinesterase was inhibited 84% and 81% in males and females at 450 mg/kg. The magnitude of these responses exhibited a dose-response relationship within each compartment. For male pups, no NOAEL was observed for cholinesterase inhibition following an acute dose; for female pups, no effects were seen at 5 mg/kg.

2. Repeated Exposures (Tables 5b and 5c)

A. Prenatal Exposures to Dams: Gestation Day 6-20 (Table 5b)

In dams on Gestation Day (GD) 20, statistically significant inhibition in red blood cell

cholinesterase was observed at 50 and 150 mg/kg/day (19 and 51%, respectively). The red blood cell cholinesterase inhibition in the GD 20 dams was similar in magnitude to that observed in adult females after 11 days of repeated dosing, namely 20 and 48%, respectively, at 50 and 150 mg/kg/day. Neither plasma nor brain cholinesterase activity was affected at any treatment level, and no effect on red blood cell cholinesterase activity was seen at 5 mg/kg/day.

In fetuses on GD 20, statistically significant inhibition in both plasma and red blood cell cholinesterase was noted at 50 and 150 mg/kg/day (14 and 15%; 11 and 19%, respectively). The magnitude of the fetal plasma cholinesterase inhibition suggests that these decreases may not be biologically significant consequences of treatment, even though these values were statistically significant at $p \leq 0.01$. At both 50 and 150 mg/kg/day, the magnitude of the red blood cell inhibition in fetuses was lower than that observed in dams. Brain cholinesterase activity was not affected at any treatment level; no effect on plasma and red blood cell cholinesterase was observed at 5 mg/kg/day.

B. 11 days of exposure - Adults (Table 5b)

At 50 and 150 mg/kg/day, statistically significant inhibition in red blood cell cholinesterase was observed in young adult males (20 and 43%, respectively) and females (20 and 48%, respectively). The magnitude of the inhibition of the red blood cell cholinesterase activity observed in these young adult males and females was generally similar to that observed in pregnant females on GD 20. At the same doses, plasma cholinesterase was decreased in males (11 and 13%, $p \leq 0.05$) and females (15 and 13%, not significant). The decreased plasma cholinesterase activity for both young adult males and females at 50 and 150 mg/kg/day were not attributed to treatment, since the magnitude of the response was not great, and a dose-response relationship was not observed in the young adult females. At 5 mg/kg/day, there was no effect of treatment on cholinesterase in any compartment; brain cholinesterase was not affected at any treatment level.

C. Prenatal and postnatal maternal exposure (Table 5b

In PND 4 pups, maternal exposure to malathion at doses up to 150 mg/kg/day resulted in no inhibition in cholinesterase activity in any compartment. A comparison of these findings with those seen for GD 20 fetuses (where plasma and/or red blood cell cholinesterase was significantly inhibited at 50 mg/kg/day and above) suggests recovery, reduction of exposure in early postnatal life, and/or differences in sampling or analysis procedures.

D. Prenatal, postnatal maternal and 11 days direct exposure (Table 5b)

In PND 21 pups, statistically significant treatment-related red blood cell cholinesterase inhibition (17%) was observed at 5 mg/kg/day in males. In female pups at 5 mg/kg/day, red blood cell cholinesterase was similarly inhibited (15%). This value was not found to be statistically significant, presumably due to the larger variance observed in the data, but was nevertheless considered to be an adverse effect of treatment. At 50 mg/kg/day, treatment-related plasma (19% in both sexes) and red blood cell (39% in males, 34% in females) cholinesterase inhibition was observed. At 150 mg/kg/day, the plasma cholinesterase inhibition was 24% and 32% for males and females, and the red blood cell inhibition was

67% and 68% for males and females. The magnitude of red blood cell inhibition at 150 mg/kg/day was greater following 11 doses, than that observed following a single dose to PND 11 pups (i.e., 55 %and 48% for males and females). Statistically significant brain cholinesterase inhibition (16%) was observed at 150 mg/kg/day for both males and females. The magnitude of brain cholinesterase inhibition observed on PND 21 after 11 repeated doses was lower than that seen after acute dosing in PND 11 pups (44 and 48% for males and females) at 150 mg/kg/day.

E. Day 60 - 40 days after exposure (Table 5c)

No statistically significant differences in plasma, red blood cell, or brain cholinesterase activity were observed at any dose for PND 60 males or females that had been exposed to malathion during development (through PND 21) and had then been allowed to recover for 39 days. Although plasma cholinesterase was decreased 23% from control in females at 150 mg/kg/day, this finding was neither statistically nor biologically significant, and was likely the consequence of an unusually high control value.

Table 5a. Plasma, RBC, and Brain Cholinesterase Activity in Adults, Fetuses, and Offspring of Rats Treated with Malathion - **Acute Exposures**

Cholinesterase	Dose (mg/kg/day)				
	0	5	50	150	450
Day 1 Adult Males (Groups 6-9) Plasma (U/L) RBC (U/L) Brain (U/kg)	342 ± 45 866 ± 168 13,713 ± 854	341 ± 36 (0) 891 ± 170 (-3) 12,988 ± 415 (5)	359 ± 67 (-5) 975 ± 84 (-13) 13,081 ± 710 (5)	337 ± 78 (1) 853 ± 60 (2) 12,744 ± 859 (7)	
Day 1 Adult Males (Groups 10-11) Plasma (U/L) RBC (U/L) Brain (U/kg)	354 ± 71 1109 ± 87 13,563 ± 392				**268** ± 36** **831*** ± 8** 13,131 ± 4
Day 1 Adult Females (Groups 6-9) Plasma (U/L) RBC (U/L) Brain (U/kg)	793 ± 216 950 ± 67 12,900 ± 471	717 ± 119 (10) 1013 ± 109 (-7) 13,213 ± 427 (-2)	822 ± 182 (-4) 959 ± 104 (-1) 13,038 ± 553 (-1)	727 ± 199 (8) 891 ± 64 (6) 13,244 ± 244 (-3)	
Day 1 Adult Females (Groups 10-11) Plasma (U/L) RBC (U/L) Brain (U/kg)	624 ± 86 1069 ± 65 13,513 ± 501				558 ± 83 **884*** ± 9** 12,975 ± 6
PND 11 Males (Offspring of Group 5) Plasma (U/L) RBC (U/L) Brain (U/kg)	756 ± 74 1509 ± 256 5756 ± 224	704 ± 52 (7) **1272* ± 239 (16)** 5688 ± 217 (1)	**614** ± 44 (19)** **1131** ± 141 (25)** 5388 ± 280 (6)	**482** ± 72 (36)** **672** ± 100 (55)** **3244** ± 699 (44)**	**346** ± 63** **428** ± 95** **919** ± 43**
PND 11 Females (Offspring of Group 5) Plasma (U/L) RBC (U/L) Brain (U/kg)	737 ± 79 1319 ± 110 5825 ± 279	717 ± 47 (3) 1228 ± 229 (7) 5600 ± 183 (4)	**620** ± 79 (16)** **1016** ± 127 (23)** **5249 ± 729 (10)**	**481** ± 46 (35)** **688** ± 58 (48)** **3044** ± 560 (48)**	**353** ± 68** **519** ± 13** **1081** ± (81)**

Data from Tables 34-41, pp. 99-112, MRID 45566201

[a] Results in parenthesis are percent inhibition relative to control

n = 8 for all measures

* p≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

Table 5b. Plasma, RBC, and Brain Cholinesterase Activity in Adults, Fetuses, and Offspring of Rats Treated with Malathion - **Repeated Exposures**

Cholinesterase	Dose (mg/kg/day)			
	0	5	50	150
GD 20 Dams (Groups 1-4) Plasma (U/L) RBC (U/L) Brain (U/kg)	1382 ± 258 1234 ± 138 13,200 ± 418	1210 ± 134 (12) [a] 1244 ± 59 (-1) 13,013 ± 659 (1)	1297 ± 173 (6) **994** ± 61 (19)** 13,100 ± 517 (1)	1204 ± 243 (13) **606** ± 75 (51)** 12,644 ± 263 (4)
GD 20 Fetuses (Groups 1-4) Plasma (U/L) RBC (U/L) Brain (U/kg)	285 ± 23 938 ± 113 1606 ± 118	265 ± 23 (7) 897 ± 69 (4) 1656 ± 178 (-3)	246** ± 39 (14) **831* ± 82 (11)** 1519 ± 173 (5)	243** ± 18 (15) **756** ± 48 (19)** 1638 ± 260 (-2)
Day 11 Adult Males (Groups 6-9) Plasma (U/L) RBC (U/L) Brain (U/kg)	333 ± 22 1084 ± 46 13,219 ± 601	322 ± 28 (3) 1044 ± 65 (4) 13,288 ± 593 (-1)	297* ± 26 (11) **869** ± 99 (20)** 13,494 ± 391 (-2)	289* ± 48 (13) **616** ± 74 (43)** 13,031 ± 724 (1)
Day 11 Adult Females (Groups 6-9) Plasma (U/L) RBC (U/L) Brain (U/kg)	1028 ± 250 1094 ± 92 13,731 ± 1857	978 ± 257 (5) 1069 ± 143 (2) 13,463 ± 319 (2)	871 ± 240 (15) **878** ± 54 (20)** 13,700 ± 464 (0)	893 ± 173 (13) **566** ± 90 (48)** 13,031 ± 442 (5)

PND 4 Male (Offspring of Groups 1-4) Plasma (U/L) RBC (U/L) Brain (U/kg)	n = 17 612 ± 59 1100 ± 170 3018 ± 270	n = 16 594 ± 66 (3) 1134 ± 155 (-3) 3078 ± 217 (-2)	n = 13 626 ± 52 (-2) 1075 ± 164 (2) 2915 ± 326 (3)	n = 15 620 ± 68 (-1) 1017 ± 197 (8) 2867 ± 420 (5)
PND 4 Female (Offspring of Groups 1-4) Plasma (U/L) RBC (U/L) Brain (U/kg)	n = 18 622 ± 59 1147 ± 193 2994 ± 265	n = 17 598 ± 62 (4) 1125 ± 190 (2) 2941 ± 317 (2)	n = 19 606 ± 52 (3) 1193 ± 246 (-4) 2953 ± 548 (1)	n = 18 617 ± 44 (1) 1103 ± 227 (4) 2967 ± 348 (1)
PND 21 Male (Offspring of Groups 1-4) Plasma (U/L) RBC (U/L) Brain (U/kg)	393 ± 75 1866 ± 394 10,500 ± 287	341 ± 76 (13) **1556* ± 282 (17)** 10,363 ± 318 (1)	**320*± 42 (19)** **1144** ± 218 (39)** 10,488 ± 506 (0)	**299** ± 48 (24)** **622** ± 207 (67)** **8850** ± 793 (16)**
PND 21 Female (Offspring of Groups 1-4) Plasma (U/L) RBC (U/L) Brain (U/kg)	374 ± 65 1894 ± 398 10,356 ± 253	338 ± 55 (10) **1606 ± 484 (15)** 10,250 ± 382 (1)	**304* ± 69 (19)** **1250** ± 160 (34)** 10,444 ± 408 (-1)	**254** ± 39 (32)** **597** ± 185 (68)** **8650** ± 931 (16)**

Data from Tables 34-41, pp. 99-112, MRID 45566201

[a] Results in parenthesis are percent inhibition relative to control

n = 8 for all measures, except PND 4 (itemized above)

* $p \leq 0.05$, ** $p \leq 0.01$

Table 5c. Plasma, RBC, and Brain Cholinesterase Activity in Adults, Fetuses, and Offspring of Rats Treated with Malathion - **Post Exposures**

Cholinesterase	**Dose (mg/kg/day)**			
	0	**5**	**50**	**150**
PND 60 Male (Offspring of Groups 1-4) Plasma (U/L) RBC (U/L) Brain (U/kg)	363 ± 49 903 ± 118 13,231 ± 524	377 ± 65 (-4) 944 ± 107 (-5) 13,269 ± 724 (0)	344 ± 42 (5) 997 ± 107 (-10) 13,125 ± 580 (1)	317 ± 72 (13) 1050 ± 281 (-16) 12,825 ± 1055 (3)
PND 60 Female (Offspring of Groups 1-4) Plasma (U/L) RBC (U/L) Brain (U/kg)	1090 ± 294 966 ± 130 13,513 ± 309	894 ± 176 (18) 988 ± 60 (-2) 13,313 ± 1138 (1)	913 ± 167 (16) 956 ± 82 (1) 13,431 ± 519 (1)	843 ± 98 (23) 994 ± 147 (-3) 13,331 ± 371 (1)

Data from Tables 34-41, pp. 99-112, MRID 45566201

[a] Results in parenthesis are percent inhibition relative to control

n = 8 for all measures

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that an acute dose of 450 mg/kg did not produce clinical signs in young adults, but resulted in body tremors 1-2 hours post-treatment for PND 11 pups, as well as a single moribund pup. Repeated doses of malathion at doses up to 150 mg/kg/day did not induce effects on body weight, body weight gain, or survival, to dams, fetuses, offspring, or adult male and female rats. Post-dose salivation was noted in dams during gestation and/or lactation at 150 mg/kg/day. Reproductive performance, gestation and implantation were not affected by treatment in dams, nor were litter size, viability, sex ratio, or post-implantation survival affected. No treatment-related effects were found at necropsy of adults or offspring, and brain weights were unaffected by treatment. Acute exposures of malathion resulted in decreased cholinesterase activity in PND 11 pups at 50 mg/kg and in young adults at 450 mg/kg. Repeated doses (11 days) of malathion resulted in decreased cholinesterase activity in dams, fetuses, PND 21 pups, and young adult male and female rats at a dose of 50 mg/kg/day. The study author did not consider the 16-17% decreases in red blood cell cholinesterase activity for male pups at PND 11 (acute dose) and PND 21 (repeated dose) to be biologically relevant in determining the NOAEL.

The study author established an overall NOAEL of 5 mg/kg/day for plasma and red blood cell cholinesterase inhibition, and 50 mg/kg/day for brain cholinesterase inhibition. The most sensitive day of treatment was determined to be at PND 11.

B. **DISCUSSION AND REVIEWER COMMENTS:** This study was conducted to determine the effects of malathion on cholinesterase activity in male and female adult, juvenile, and fetal rats following oral administration.

An acute 450 mg/kg dose of malathion resulted in tremors in 5 of 16 PND11 pups at 1-2 hours post-treatment, as well as moribundity in one pup; no clinical observations were noted in young adults at this acute 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 clinical observations, survival, body weight, body weight gain, brain weight, or gross pathology in young adult male and female rats, juveniles, or fetuses. (In the initial range-finding study, 450 and 200 mg/kg/day administered to offspring elicited such severe toxicity as to require termination for humane reasons. These findings in offspring served as the rationale for conducting the subsequent multiple dosing cholinesterase and DNT studies at the top dose level of 150 mg/kg/day.) Additionally, reproductive performance, gestation length, sex ratio, and pre- and postnatal viability were unaffected by treatment.

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 young adults, a single gavage dose of malathion resulted in inhibition of plasma (males 24%) and red blood cell (males 25%, females 17%) cholinesterase at 450 mg/kg, while after 11 or 14 consecutive repeated gavage doses, red blood cell inhibition was observed in young adults or pregnant dams at 50 mg/kg/day (19-20%) and 150 mg/kg/day (43-51%), but plasma and brain cholinesterase were not inhibited at any dose. In PND 11 pups, a single gavage dose of malathion resulted in red blood cell inhibition in males (16%, 25%, 55% and 72% at 5, 50, 150, and 450 mg/kg,

respectively) and in females (23%, 48%, and 61% at 50, 150 and 450 mg/kg, respectively); plasma cholinesterase inhibition in males (19%, 36%, and 54%) and females (16%, 35%, and 52%) at 50, 150, and 450 mg/kg, respectively; and brain cholinesterase inhibition in males (10%, 44% and 84% at 50, 150, and 450 mg/kg, respectively) and females (48% and 81% at 150 and 450 mg/kg, respectively). In PND 21 pups, 11 repeated gavage doses from PND 11-21 resulted in red blood cell cholinesterase inhibition in males (17%, 39%, and 67%) and females (15%, 34%, and 68%) at 5, 50, and 150 mg/kg/day, respectively; plasma cholinesterase inhibition in males (19% and 24%) and females (19% and 32%) at 50 and 150 mg/kg/day; and brain cholinesterase inhibition in males (16%) and females (16%) at 150 mg/kg/day. By PND 60 (39 days after the last repeated dose), cholinesterase activity in offspring (all compartments) was similar between control and treated groups.

The statistically significant effects of acute and repeated exposures on red blood cell cholinesterase activity at 5 mg/kg/day for male pups are considered by Agency reviewers to be biologically significant treatment-related effects, although these findings were dismissed by the study author. The red blood cell cholinesterase findings in male pups demonstrate a clear dose-dependant relationship, both for acute and repeated exposures. The percent red blood cell cholinesterase inhibition ($p \leq 0.05$) for PND 11 male pups following acute exposure is 16%, 25%, 55%, and 72% for the 5, 50, 150, and 450 mg/kg dose groups, respectively. For the repeated dose PND 21 males, the percent red blood cell cholinesterase inhibition ($p \leq 0.05$) is 17%, 39%, and 67% for the 5, 50, and 150 mg/kg/day dose groups. While statistical significance was not observed for cholinesterase activity values in PND 21 female pups at 5 mg/kg/day following repeated exposures, the percent inhibition of red blood cell cholinesterase activity was 15%, which is notably similar to the magnitude of inhibition observed in males (as well as being similar in terms of dose response - 15%, 34%, and 68% for the 5, 50, and 150 mg/kg/day dose groups) and supports the interpretation of these findings as treatment-related. This conclusion is also supported by the fact that red blood cell cholinesterase was the compartment consistently inhibited at the lowest adverse effect level in every population and dosing scenario tested in this study.

The Agency reviewers agreed with the study author that 11 days of repeated dosing did not adversely affect plasma cholinesterase at any dose in adults. The magnitude of the response in males and females at those doses were remarkably similar: 11% and 13% inhibition in males ($p \leq 0.05$) at 50 and 150 mg/kg/day versus 15% and 13% inhibition in females at the same doses. There appears to be a high level of variance in the plasma cholinesterase measures for adult females at these doses. Nevertheless, the magnitude of the response and the lack of a dose-response relationship for females suggest that these findings are not treatment-related. Likewise, the Agency reviewers agree with discounting the 13% plasma cholinesterase inhibition among male offspring at 5 mg/kg/day. This interpretation of the data is supported by the plasma cholinesterase data in GD 20 dams, where nonsignificant decreases of 6% and 13% from control were noted at 50 and 150 mg/kg/day, and in GD 20 fetuses, where significant decreases of 14% and 15% were observed at the same doses. These findings in dams and fetuses at GD 20 were also not judged to be adverse consequences of treatment, although it is recognized that the plasma cholinesterase inhibition values in fetuses were significant at $p \leq 0.01$.

A decrease (10%) in brain cholinesterase activity at 50 mg/kg in PND 11 females following an acute exposure of malathion was considered to be biologically significant and treatment-

related by the Agency reviewers, even though this finding was not statistically significant. It is believed that this represents a threshold response to treatment. The magnitude of the inhibition supports this opinion; significant findings of 5-10% or so for brain cholinesterase inhibition are not uncommon, based on the lower variability usually seen in this tissue in comparison in general to the blood measures. Additionally, a noteworthy dose-response was observed in the brain cholinesterase data, culminating in 81% inhibition for females at 450 mg/kg. The magnitude of the response in males (6% less than control) was considered more equivocal in interpretation, even though there was a similar dose response as seen in the females.

The cholinesterase activity measures following acute or repeated gavage doses of malathion in this study, demonstrate that juvenile rats are more sensitive than adults. Both for PND 11 pups that were evaluated after a single dose of malathion, and for PND 21 pups that were evaluated after 11 consecutive days of repeated dosing with malathion, cholinesterase was inhibited in all compartments (plasma, RBC, and brain) at lower doses than adults. With both dosing scenarios, the pup NOAEL for plasma cholinesterase inhibition was 5 mg/kg/day. The male pup NOAEL was not identified for red blood cell inhibition, since significant inhibition was noted at 5 mg/kg (the lowest dose tested) following either acute or repeated doses; for female pups, the red blood cell NOAEL was identified at 5 mg/kg for acute exposures and was <5mg/kg/day for repeated exposures. However, in adults, the NOAEL for plasma cholinesterase inhibition following a single dose was 150 mg/kg in males and 450 mg/kg in females; following repeated doses, the adult plasma cholinesterase inhibition NOAEL was ≥450 mg/kg/day for males and females. The adult NOAEL for red blood cell cholinesterase inhibition following repeated doses was at 5 mg/kg/day in both males and females. The pup NOAEL for brain cholinesterase inhibition was 5 mg/kg for males and 50 mg/kg for females following an acute dose at PND 11, and it was 50 mg/kg/day on PND 21 following repeated doses for both sexes. In adults, the brain cholinesterase NOAEL was established at ≥450 mg/kg (the highest dose tested) for acute doses and ≥150 mg/kg/day (also the highest dose tested) following repeated doses. Additionally, when inhibition was noted for both age groups at the same dose level, the magnitude of the response, i.e., the percent inhibition as compared to control, was substantially greater for pups than for young. For example, after an acute dose of 450 mg/kg, red blood cell cholinesterase inhibition in adult males and females was 25% and 17%, respectively, while for PND 11 pups it was 72% for males and 61% for females. Similarly, after 11 days of repeated dosing at 150 mg/kg/day, red blood cell cholinesterase inhibition in adult males and females was 43% and 48%, respectively, while for PND 21 pups it was 67% for males and 68% for females. The remarkable inhibitions of the brain enzyme of 81-85% at 450 mg/kg/day and 44-48% at 150 mg/kg/day in immature rats, versus no brain cholinesterase inhibition in adults at these doses, is particularly noteworthy as evidence of enhanced vulnerability of the CNS in the developing individual.

The Agency and the study author agree that the most sensitive age of treatment in this study was PND 11. Following an acute dose of 150 mg/kg in PND 11 pups, brain cholinesterase was inhibited 44% in males and 48% in females. In PND 21 pups, after 11 days of repeated dosing at 150 mg/kg/day, brain cholinesterase was inhibited 16% in males and females. In young adu tested (450 mg/kg after an acute dose, and 150 mg/kg/day after 11 days of repeated dosing). The inhibition in offspring at 150 mg/kg/day of 44-48% (single dose) at PND 11, as contrasted with 16% (repeated dosing) by PND 21, suggests decreasing vulnerability of the CNS enzyme with increasing age of the offspring. Such evidence elicits a concern that at yet

earlier time points in the PND 1-11 period, vulnerability of the CNS may be greater than at PND 11. In support of this, it is noted that while red blood cell cholinesterase inhibition increased in offspring with repeated dosing at 150 mg/kg/day as one might expect [inhibition 48-55% at PND 11 (single dose); 67-68% at PND 21 (repeated dosing)], brain cholinesterase inhibition decreased between PND 11 and PND 21. This divergence between red blood cell cholinesterase inhibition and brain cholinesterase inhibition with increasing age, suggests a progressive enhancement of the level of protection of the CNS enzyme with increasing age, again raising a question as to the enzyme vulnerability in the PND 1-11 period.

In another example, following an acute dose of 50 mg/kg, red blood cell cholinesterase was inhibited in PND 11 pups (25% in males, 23% in females); following repeated doses of 50 mg/kg/day, red blood cell cholinesterase was also inhibited in PND 21 pups (39% in males, 34% in females). However, in adults, an acute dose of 50 mg/kg resulted in no red blood cell cholinesterase inhibition, while after 11 days of repeated dosing at 50 mg/kg/day, red blood cell cholinesterase was inhibited 20% in both sexes. The difference in response between adults and pups at PND 11 is much greater than at PND 21. This comparison suggests that had even younger pups (e.g., PND 1, 4, or 7) been administered direct doses of malathion, an even greater differential in response between offspring and adults might have been noted, characterized by a greater magnitude of red blood cell inhibition in offspring at 5 mg/kg/day on PND 11. This age-related sensitivity is biologically plausible and could be due to the normal ontogeny of the carboxylesterase enzymes and/or to the maturation of the blood brain barrier.

GD 20 fetuses were not more sensitive to the cholinesterase-inhibiting effects of malathion than were their dams, following maternal exposure from GD 6-20. The red blood cell cholinesterase NOAEL in each case was at 5 mg/kg/day, and dams showed greater red blood cell cholinesterase inhibition than fetuses (19% versus 11%) at 50 mg/kg/day.

The data from this study demonstrate no statistically or biologically significant decreases in plasma, red blood cell, or brain cholinesterase activity for PND 4 pups, in samples collected 4 hours after maternal treatment at 5, 50, and 150 mg/kg/day. (The dams of these PND 4 pups had been treated continuously at these dose levels since GD 6.) However, in GD 20 fetuses (killed 3 hours after dosing), plasma and red blood cell cholinesterase was significantly inhibited (between 11 and 19% of control) at 50 and 150 mg/kg/day. The apparent recovery in cholinesterase activity by PND 4 under these study conditions may be due to 1) reduced exposure in early postnatal life, that is, an absence of malathion or its oxon metabolite in the milk, 2) more rapid recovery, or 3) differences in the timing of sampling in relation to exposure.

<u>For acute exposures</u>:

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).

C. STUDY DEFICIENCIES:

The study report did not include clinical observation or mortality data for directly dosed pups from PND 11-21. The registrant is asked to provide this information.

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No justification was provided for the time of blood sampling in relation to dosing, e.g., time-to-peak-effect data for adults and pups. The registrant is asked to provide this information.

DATA FOR ENTRY INTO ISIS

Special Study

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
0057701	45566201	special ChE study	rats	Acute dose (1 day)	oral	gavage	0-450	0, 5, 50, 150, 450	150	450	Cholinesterase activity inhibition (plasma, RBC)	Adult
0057701	45566201	special ChE study	rats	Repeated dose (11 days)	oral	gavage	0-150	0, 5, 50, 150	5	50	Cholinesterase activity inhibition (RBC)	Adult
0057701	45566201	special ChE study	rats	Acute dose (1 day)	oral	gavage	0-450	0, 5, 50, 150, 450	<5	5	Cholinesterase activity inhibition (RBC)	Offspring (PND 11)
0057701	45566201	special ChE study	rats	Repeated dose (11 days)	oral	gavage	0-150	0, 5, 50, 150	<5	5	Cholinesterase activity inhibition (RBC)	Offspring (PND 21)

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