TXR No. 0050550

MEMORANDUM

DATE: August 22, 2002

SUBJECT: D282204: Malathion (057701) Developmental neurotoxicity data reviews:
1) developmental neurotoxicity study in rats (MRID 45646401; D282204)
2) comparative cholinesterase inhibition study in adult and juvenile rats (MRID 45566201, D280071)
3) dose range-finding developmental neurotoxicity study in rats (MRID 45627001; D281554)

TO: Anne Overstreet
Chemical Review Manager
Special Review and Reregistration Division (7508C)

FROM: Susan L. Makris   *[signature]* 8/22/02
Toxicology Branch
Health Effects Division (7509C)

and
Brian Dementi   *[signature]* 8/22/02
Toxicology Branch
Health Effects Division (7509C)

THRU: Alberto Protzel, Branch Senior Scientist   *[signature]* 8/23/02
Toxicology Branch
Health Effects Division (7509C)

CC: Paula Deschamp (7509C)

ACTION REQUESTED: Review the following toxicology studies, which were submitted in response to the 1999 Data-Call-In for a developmental neurotoxicity study with malathion (057701):
1. Developmental neurotoxicity study in rats (MRID 45646401)
2. Comparative cholinesterase study in adult and juvenile rats (companion study to DNT) (MRID 45566201)
3. Dose range-finding developmental neurotoxicity study in rats (MRID 45627001)

CONCLUSION: The submitted studies satisfy the requirements of the 1999 Data-Call-In for a developmental neurotoxicity study in rats with malathion. Executive summaries are presented below:

1. Developmental Neurotoxicity Study in Rats

Citation: Fulcher, S. M. (2002) Malathion. Developmental neurotoxicity study in the CD rat by oral gavage administration. Huntington Life Sciences, Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. Laboratory report number CHV/066; 013331, March 21, 2002. MRID 45646401. Unpublished

Executive Summary: In a developmental neurotoxicity study (MRID 45646401), malathion (96% a.i., batch # 9010501) was administered to 24 parental female Crl:CD®BR rats per dose by gavage at dose levels of 0, 5.0, 50, or 150 mg/kg bw/day in corn oil from gestation day 6 through postnatal day 10, and to the offspring from postnatal day 11 to postnatal day 21 inclusive. A Functional Observational Battery was performed on 10 dams/dose on gestation days 12 and 18 and lactation days 4 and 10. Offspring were evaluated as follows: age-appropriate functional observation battery on days 4, 11, 21, 35, 45, and 60, automated motor activity on days 13, 17, 22, and 60; assessment of auditory startle response on days 23/24 and 60/61, assessment of learning and memory (Morris Water Maze) at postnatal days 23/24, and at postnatal day 61/62 (separate groups), brain weights on days 11, 21, and 65, and brain histopathology and morphometrics on days 21 and 65. Pup physical development was assessed by body weight. Sexual maturation of females was assessed by age of vaginal opening, and sexual maturation of males was assessed by age at completion of balano-preputial separation.

There were no treatment-related maternal deaths before scheduled termination. Clinical signs were limited to transient post-dosing salivation (5/24 control, 4/24 at 5 mg/kg/day, 3/24 at 50 mg/kg/day, and 20/24 at 150 mg/kg/day). There were no other treatment-related effects on cholinergic signs, and there were no effects on maternal body weight, food consumption, or reproductive indices. **The maternal LOAEL for malathion in rats is 150 mg/kg/day based on an increased incidence of post-dosing salivation. The maternal NOAEL is 50 mg/kg/day.**

The **offspring NOAEL is <5 mg/kg/day** (the lowest dose tested). The **offspring LOAEL is 5 mg/kg/day, based upon increased auditory startle reflex peak amplitude in PND 23/24 male and female offspring and decreased habituation in PND 60/61 females.** At 50 mg/kg/day, there was an increased incidence of slightly flattened gait in PND 60 males, and motor activity counts (rearing and ambulatory) were decreased in female pups at PND 17 and 22. At 150 mg/kg/day, additional treatment-related findings included post-dosing clinical observations on PND 17 and 18 (whole body tremors, hypoactivity, prostrate posture, partially closed eyelids, and/or abnormal gait), delayed surface righting reflex in PND 11 female pups, increased incidences of slightly flattened gait in PND 60 males, and increased thickness of the corpus callosum in PND 63-67 males and females.

In a companion cholinesterase inhibition study (MRID 45566201), 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 on RBC cholinesterase were noted at 5 mg/kg in males and 50 mg/kg in females

following single dose acute exposures on PND 11. and at 5 mg/kg/day in both sexes on PND 21 after 11 repeated exposures. Following a single dose to young adults. effects on RBC cholinesterase 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, brain cholinesterase was inhibited at 150 mg/kg/day following an acute dose (44-48%) in PND 11 pups or after 11 repeated doses (16% in PND 21 pups). Based upon the results of the cholinesterase study. it is evident that all behavioral and neuropathological effects of treatment observed in the dams and offspring in the developmental neurotoxicity study occurred at doses at which cholinesterase was, or had been, inhibited. For acute and repeated exposures the overall LOAEL for cholinesterase inhibition was 5 mg/kg/day, based on RBC cholinesterase inl..bition in PND 11 and 21 pups. The NOAEL was not determined.

This study is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6).

2. Comparative Cholinesterase Study in Adult and Juvenile Rats

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

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

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

However, acute or repeated exposure to malathion resulted in statistically and biologically significant decreases in cholinesterase activity in the blood and/or brain in dams, fetuses, weanling pups, and adult male and female rats.. In pups, effects were noted at 5 mg/kg in males and 50 mg/kg in females following single dose acute exposures, and at 5 mg/kg/day in both sexes

after repeated exposures. Following a single dose to young adults, effects were observed at 450 mg/kg, while after 11 or 14 doses, effects were observed at 50 mg/kg/day in young adults and pregnant dams. In pups, effects were noted at 5 mg/kg/day in males and 50 mg/kg/day in females following single dose acute exposures, and at 5 mg/kg/day in both sexes after repeated exposures. By PND 60 (39 days after the last dose), cholinesterase activity levels in offspring were similar between control and treated groups.

For acute exposures:

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

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

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

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

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

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

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

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

For repeated exposures:

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

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

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

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

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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.

3. Dose Range-Finding Developmental Neurotoxicity Study in Rats

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

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

determined in maternal and fetal plasma, RBC, and brain. Treated offspring were killed two hours after dosing on postnatal day 21 and cholinesterase activities determined.

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

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

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

This study is classified **Acceptable/Nonguideline** as a dose range-finding study and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6).

EPA Reviewer: Susan Makris   Signature _____
Toxicology Branch, Health Effects Division (7509C)   Date __4/23/02__

EPA Reviewer: Brian Dementi   Signature _____
Toxicology Branch, Health Effects Division (7509C)   Date __4/23/02__

TXR#: 0050550

<div style="text-align:center">

DATA EVALUATION RECORD

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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.

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

For acute exposures:

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

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

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

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

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

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

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

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

For repeated exposures:

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

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

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

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

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

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

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

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

The cholinesterase activity measures following acute or repeated gavage doses of malathion in this study, demonstrate that juvenile rats are more susceptible than adults. Overall, this susceptibility was observed in terms of the dose level at which effects were observed (i.e., the NOAELs for cholinesterase inhibition were lower for juveniles than for adults), the compartments in which a response was elicited (e.g., brain cholinesterase was inhibited in offspring but was not observed in adults up to the highest dose tested), and the magnitude of the response (i.e., when inhibition was noted for both age groups at the same dose level, the percent inhibition was substantially greater for pups than for young adults). This same susceptibility was not demonstrated for GD 20 fetuses when compared to dams, following maternal exposure from GD 6-20.

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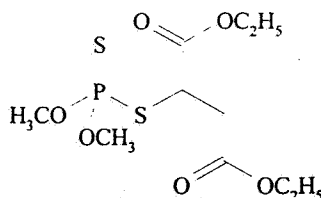
2. **Vehicle and/or positive control:** corn oil was used as the negative control vehicle in this study; no positive control substance was tested

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 g, 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 for 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 12-15 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.

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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
11	450	8F/8M	Eight males and females were treated for one day. a

Data from pp 24-25 of MRID 45566201
a Animals received a..d placed on study after the dosing of the other young adult rats (Groups 6-9) had been completed.

3. **Mating procedure:** Females were paired on a 1:1 basis with stock males of the same strain. 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.

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

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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.

b. **Offspring:** The day of completion of parturition was designated as lactation day (post-natal 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

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| 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 p. :ked 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

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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.

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 Day 4 (precull) Day 11	15.0 ± 1.6 14.7 ± 1.8 8.0 ± 0.0	15.7 ± 1.8 15.0 ± 2.4 8.0 ± 0.0	13.9 ± 1.6 13.8 ± 1.5 8.0 ± 0.0	14.2 ± 1.9 14.1 ± 2.0 8.0 ± 0.0
Pup Deaths Birth (Stillborn) Days 1-4 Days 5-11	0 3 0	0 7 0	0 1 0	0 1 0
Live Birth Index (%)	100	100	100	100
Viability Index (%)	97.9	95.2	99.4	99.2

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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 ≤ 0.05, ** p ≤ 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)	0.407 ± 0.043	0.396 ± 0.043	0.414 ± 0.060	0.421 ± 0.036	
Female (n = 18, 17, 19, 18)	0.400 ± 0.044	0.386 ± 0.038	0.416 ± 0.044	0.414 ± 0.043	
PND 11 (Group 5) (n = 8)					
Male	1.008 ± 0.051	0.993 ± 0.049	1.019 ± 0.034	1.021 ± 0.059	0.998 ± 0.046
Female	0.980 ± 0.047	0.984 ± 0.057	0.994 ± 0.084	0.971 ± 0.060	1.004 ± 0.078
PND 21 (n = 8)					
Male	1.493 ± 0.063	1.457 ± 0.069	1.520 ± 0.080	1.479 ± 0.057	
Female	1.471 ± 0.043	1.429 ± 0.077	1.419 ± 0.053	1.428 ± 0.063	
PND 60 (n = 8)					
Male	2.016 ± 0.090	2.003 ± 0.081	1.990 ± 0.047	1.975 ± 0.062	
Female	1.862 ± 0.066	1.852 ± 0.051	1.868 ± 0.108	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)

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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≤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 (24) 831*** ± 85 (25) 13,131 ± 451 (3)
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 (11) 884*** ± 96 (17) 12,975 ± 639 (4)
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 (54) 428** ± 95 (72) 919** ± 435 (84)
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 (52) 519** ± 135 (61) 1081** ± 513 (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
* $p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

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)

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Cholinesterase	Dose (mg/kg/day)			
	0	5	50	150
Day 11 Adult Males (Groups 6-9)				
Plasma (U/L)	333 ± 22	322 ± 28 (3)	297* ± 26 (11)	289* ± 48 (13)
RBC (U/L)	1084 ± 46	1044 ± 65 (4)	869** ± 99 (20)	616** ± 74 (43)
Brain (U/kg)	13,219 ± 601	13,288 ± 593 (-1)	13,494 ± 391 (-2)	13,031 ± 724 (1)
Day 11 Adult Females (Groups 6-9)				
Plasma (U/L)	1028 ± 250	978 ± 257 (5)	871 ± 240 (15)	893 ± 173 (13)
RBC (U/L)	1094 ± 92	1069 ± 143 (2)	?78** ± 54 (20)	566** ± 90 (48)
Brain (U/kg)	13,731 ± 1857	13,463 ± 319 (2)	13,700 ± 464 (0)	13,031 ± 442 (5)
PND 4 Male (Offspring of Groups 1-4)	n = 17	n = 16	n = 13	n = 15
Plasma (U/L)	612 ± 59	594 ± 66 (3)	626 ± 52 (-2)	620 ± 68 (-1)
RBC (U/L)	1100 ± 170	1134 ± 155 (-3)	1075 ± 164 (2)	1017 ± 197 (8)
Brain (U/kg)	3018 ± 270	3078 ± 217 (-2)	2915 ± 326 (3)	2867 ± 420 (5)
PND 4 Female (Offspring of Groups 1-4)	n = 18	n = 17	n = 19	n = 18
Plasma (U/L)	622 ± 59	598 ± 62 (4)	606 ± 52 (3)	617 ± 44 (1)
RBC (U/L)	1147 ± 193	1125 ± 190 (2)	1193 ± 246 (-4)	1103 ± 227 (4)
Brain (U/kg)	2994 ± 265	2941 ± 317 (2)	2953 ± 548 (1)	2967 ± 348 (1)
PND 21 Male (Offspring of Groups 1-4)				
Plasma (U/L)	393 ± 75	341 ± 76 (13)	320* ± 42 (19)	299** ± 48 (24)
RBC (U/L)	1866 ± 394	1556* ± 282 (17)	1144** ± 218 (39)	622** ± 207 (67)
Brain (U/kg)	10,500 ± 287	10,363 ± 318 (1)	10,488 ± 506 (0)	8850** ± 793 (16)
PND 21 Female (Offspring of Groups 1-4)				
Plasma (U/L)	374 ± 65	338 ± 55 (10)	304* ± 69 (19)	254** ± 39 (32)
RBC (U/L)	1894 ± 398	1606 ± 484 (15)	1250** ± 160 (34)	597** ± 185 (68)
Brain (U/kg)	10,356 ± 253	10,250 ± 382 (1)	10,444 ± 408 (-1)	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≤ 0.05, **p ≤ 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)	363 ± 49	377 ± 65 (-4)	344 ± 42 (5)	317 ± 72 (13)
RBC (U/L)	903 ± 118	944 ± 107 (-5)	997 ± 107 (-10)	1050 ± 281 (-16)
Brain (U/kg)	13,231 ± 524	13,269 ± 724 (0)	13,125 ± 580 (1)	12,825 ± 1055 (3)
PND 60 Female (Offspring of Groups 1-4)				
Plasma (U/L)	1090 ± 294	894 ± 176 (18)	913 ± 167 (16)	843 ± 98 (23)
RBC (U/L)	966 ± 130	988 ± 60 (-2)	956 ± 82 (1)	994 ± 147 (-3)
Brain (U/kg)	13,513 ± 309	13,313 ± 1138 (1)	13,431 ± 519 (1)	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

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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 adult males and females, brain cholinesterase was not inhibited at the highest dose 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), 57-68% 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.

For acute exposures:

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

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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. <u>STUDY DEFICIENCIES</u>:

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.

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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DATA EVALUATION RECORD

MALATHION/057701

Study Type: DOSE-FINDING DEVELOPMENTAL NEUROTOXICITY
[NON-GUIDELINE]
MRID 45627001

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 02-30

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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DATA EVALUATION RECORD
TXR#: 0050550

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STUDY TYPE: Dose Range-Finding Developmental Neurotoxicity Study - Rat; Non-guideline

PC CODE: 057701

DP BARCODE: D281554
SUBMISSION NO.: S612070

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

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

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

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

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

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

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

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

This study is classified **Acceptable/Nonguideline** as a dose range-finding study and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6).

COMPLIANCE: Signed and dated GLP, Flagging, and Data Confidentiality statements were provided. A Quality Assurance statement was not included. It was noted that while the study generally followed GLP principles, no specific study-related Quality Assurance procedures or analysis of dose formulations were performed.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:**	Malathion
Description:	Pale, yellowish solid
Batch #:	9010501
Purity:	96 % a.i.
Compound Stability:	Stable for at least 1 year from date of analysis when stored in refrigerator (a Certificate of Analysis, including stability and storage conditions, was provided)
CAS # of TGAI:	121-75-5

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Structure:
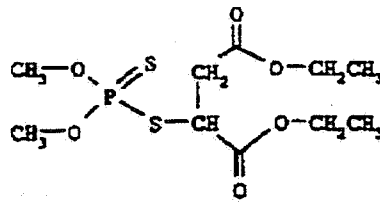
2. **Vehicle and/or positive control:** Corn oil was used as the vehicle and negative control. No positive control was used in this study.

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

Species:	Rat
Strain:	Crl:CD* BR
Age at study initiation:	10-12 wks (Phase 1); 10-11 weeks (Phase 2)
Wt. at study initiation:	207-277 g (Phase 1); 215-253 g (Phase 2)
Source:	Charles River UK Limited, Margate, Kent, England
Housing:	In stainless steel or high density polypropylene suspended cages; wood shavings provided as bedding from late gestation onwards
Diet:	Pelleted rodent diet, UAR VRF1 Certified, Usine d' Alimentation Rationale, *ad libitum*
Water:	Tap water, *ad libitum*

Environmental conditions:		
	Temperature:	19-25°C
	Humidity:	40-70%
	Air changes:	Positive pressure to outside with fresh filtered air, not-recirculated
	Photoperiod:	12 hrs dark/12 hrs light

Acclimation period:	10 days (Phase 1); 5 days (Phase 2)

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Phase 1 - Start: May 29, 2000 (initiation of mating); End: July 14, 2000 (last day of necropsy); Phase 2 - Start: August 7, 2000 (initiation of mating); End: September 25, 2000 (last day of necropsy)

2. **Study schedule:** In Phase 1 of the study, ten maternal animals/group were administered the test substance from gestation day (GD) 6 through postnatal day 10; an additional five dams/group were dosed on GD 6-20, inclusive. Following mortalities at 1250 mg/kg/day during the first four days of treatment, the dose for this group was reduced to 1000 mg/kg/day. Thereafter, mortality was also observed at 1000 and 750 mg/kg/day during late gestation; dosing was stopped for these groups (on GD 16-19) and survivors were sacrificed on GD 20. Controls and rats in the 7.5 mg/kg/day groups from the same Phase 1 study were treated as scheduled. In addition, offspring (2/sex/litter) from the control and 7.5 mg/kg/day groups were tested at 200 and 450 mg/kg/day, respectively. This was considered necessary as an effort to salvage information from Phase 1 after 1250, 1000, and 750 mg/kg/day proved to be excessively toxic to dams, requiring suspension of dosing in this higher dosage range. The 200 and 450 mg/kg/day dosage levels proved to be excessively toxic to pups, also necessitating the suspension of dosing in these offspring. In the second phase of the study, 10

maternal animals/group were administered the test substance from gestation day (GD) 6 through postnatal day 10; an additional five dams/group were dosed on GD 6-20, inclusive at doses of 0, 7.5, 35, 75, or 150 mg/kg/day. In both phases, two male and two female pups/litter were treated from postnatal day 11 to 21, inclusive. For Phase 1, an additional 2 male and 2 female pups/litter (from control dams or dams treated at 7.5 mg/kg/day) were also dosed from PND 11 at 200 or 450 mg/kg/day. However, these doses were too high and dosing was terminated and pups sacrificed before reaching weaning. Measurements of plasma, RBC, and brain cholinesterase activities were successfully obtained from pups treated at 200 mg/kg/day. In both phases, the females treated up to GD 20 were killed three hours after dosing on that day; litter data were assessed and cholinesterase activity determined in maternal and fetal plasma, RBC, and brain. For both phases, surviving treated offspring were killed two hours after dosing on postnatal day 21 and cholinesterase activities determined. Additionally, for Phase 2 plasma, RBC, and brain cholinesterase activities were determined for one male and one female dosed offspring per control litter on PND 15 to provide data for comparison with offspring at 200 mg/kg/day (Phase 1).

3. **Mating procedure:** Females were paired 1:1 with stock males of the same strain. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day 0.

4. **Animal assignment:** Mated females were allocated to group and cage position in sequence, thus ensuring that animals mated on any one day were evenly distributed among the groups (Tables 1a and 1b). The allocation of females was adjusted to avoid a given male having mated with more than one female in a study group.

TABLE 1a. Study design				
Phase 1				
Treatment schedule	**Dose (mg/kg/day)**			
	0	**7.5**	**750**	**1250/1000 a**
	Maternal Animals (n)			
GD 6-20 (cholinesterase determinations)	5	5	5	5
GD 6 - postnatal day 10	10	10	10	10
Treatment schedule	**Dose (mg/kg/day)**			
	0	**7.5 b**	**200 c**	**450 b**
	Offspring (n)			
Postnatal day 11-21 (cholinesterase determinations) - 2/sex/litter	20	20	20	20

TABLE 1b. Study design					
Phase 2					
Treatment schedule	Dose (mg/kg/day)				
	0	7.5	35	75	150
	Maternal Animals (n)				
GD 6-20 (cholinesterase determinations)	5	5	5	5	5
GD 6 - postnatal day 10	10	10	10	10	10
Treatment schedule	Dose (mg/kg/day)				
	0	7.5	35	75	150
	Offspring (n)				
Postnatal day 11-21 (cholinesterase determinations) - 2/sex/litter	20	20	20	20	20

Data taken from text table pp. 19-20, MRID 45627001.
a Dosage reduced from 1250 mg/kg/day to 1000 mg/kg/day on GD 8-11, due to excessive toxicity.
b Derived from dams treated at 7.5 mg/kg/day.
c Derived from control dams.

5. **Dose selection rationale**: For Phase 1, dose levels were chosen by the sponsor based on available toxicity data. For Phase 2, dose levels were based on results obtained in Phase 1.

6. **Dosage administration**: All doses were administered once daily to maternal animals by gavage, on either GD 6-20 or GD 6 through postnatal day 10. Two offspring/sex/litter were treated on postnatal days 11-21. Dosing volumes for dams and offspring were 5 mL/kg of body weight/day. For dams, dosing was based on the most recent body weight determination up to and including GD 17; thereafter dosing remained constant to postnatal day 1. Dams were not dosed during the parturition process. From postnatal day 1, doses were again based on the most recently recorded body weight. For offspring, doses were based on the most recently recorded body weight.

7. **Dosage preparation and analysis**: Formulations were initially prepared on a daily basis; preparation was changed to a weekly basis when sufficient stability data became available. For each concentration, the required amount of malathion was weighed out and mixed with a small amount of corn oil. The formulations were then thoroughly mixed and brought up to the required volume using corn oil.

Results:

Analytical procedure: The analytical procedure was validated for malathion in corn oil with respect to the specificity of the chromatographic analysis, linearity of detector response, precision of injection, method accuracy, and precision in relation to the conduct of the developmental neurotoxicity study (MRID 45646401).

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 45646401, 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: For the first and last weeks of the dosing period, single samples (4 x 1 mL) were taken from all groups being dosed at that time. Two assays were taken from each sample for concentration analysis. Concentrations of the dosing solutions ranged from 88.7% to 99.3% of nominal. All analytical results fell within ±10% of nominal, except for the results for the 7 mg/ml dose formulation during the last week of study; the mean concentration at that time was within ±12% of nominal. This amount of variability is generally considered acceptable. In conclusion, the analytical data indicated that the mixing procedures were adequate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** All animals were checked at least twice daily for clinical signs or ill health. Additional, detailed observations were made at six intervals on each treatment day. Signs of toxicity were recorded as they were observed, including the time of onset, degree, and duration. A standard arena observation was conducted once during the Phase 1 late gestation period, approximately 4 hours after dosing. No other functional observational battery (FOB) testing was conducted.

Individual maternal body weight data were recorded on GD 0, 3, 6, 10, 14, 17, and 20 and on lactation days 1, 4, 11, 17, and 21. Food consumption was recorded on GD 0-2, 3-5, 6-9, 10-13, 14-16, and 17-19 and on lactation days 1-3, 4-6, 7-10, 11-13, 14-16, and 17-20.

b. **Offspring/Litter observations:** The day of completion of parturition was designated as lactation day (postnatal day) 0. The females allocated to litter were allowed to deliver their young naturally and rear their own offspring until lactation day 21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity, changes in litter size, and clinical signs of toxicity. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. Additional, detailed observations were made on each treatment day. Sex of the offspring was determined on lactation days 1, 4, and 21 and individual body weights were recorded on days 1, 4, 7, and 11-21.

For Phase 1, on day 4 postpartum, litters were standardized to a maximum of 10 pups/litter (5/sex/litter, as nearly as possible). This procedure allowed two separate dosages to be investigated while still retaining undosed offspring for comparison of sibling performance. For Phase 2, with the exception of the controls, litters were culled to 8 pups/litter (4/sex/litter as nearly as possible) on PND 4. This procedure allowed collection of control cholinesterase data for offspring at PND 15 for comparison with data collected for offspring at 200

mg/kg/day in Phase 1 while still retaining undosed offspring for comparison of sibling performance.

Offspring were not evaluated for developmental landmarks, FOB, motor activity, auditory startle reflex habituation, or learning and memory.

2. **Cholinesterase determination:** Cholinesterase activity was determined in blood and brain samples from dams and fetuses on GD 20 and from two male and two female pups/litter which were dosed on lactation days 11-21. Blood was collected under light isoflurane anesthesia from the retro-orbital sinus (dams and 21-day old pups) or umbilical cord (GD 20 fetuses). Samples for GD 20 dams and fetuses were collected 3 hours post-dosing and pooled separately for male and female fetuses in each litter. (Dosing had already been suspended for the dams treated with 750 and 1250/1000 mg/kg/day, and blood samples were not collected in relation to the time of dosing. All animals at these doses were allocated to GD 20 necropsy and cholinesterase sampling was limited to all adults and 5 litters/group). Blood samples were obtained from 21-day old pups 2 hours post-dosing. Following blood collection, the brains were removed and weighed. Plasma, RBC, and whole brain cholinesterase activity was determined by a modified Ellman method, using a Hitachi 911 analyzer.

For Phase 2 dams at GD 20 and Phase 2 dosed neonates at PND 21, the following biochemical parameters were also measured: sodium, potassium, bicarbonate, chloride, calcium, and magnesium.

3. **Postmortem observations:**

. a. **Maternal animals:** On GD 20, maternal animals were sacrificed after blood sampling by carbon dioxide inhalation and subjected to a detailed macroscopic necropsy. The reproductive tract was examined for numbers of corpora lutea, implantation and resorption sites, and number and distribution of fetuses. Brains were removed and weighed. Dams that had been allowed to litter were sacrificed on or about lactation day 21 and examined grossly; the number of implantation sites was recorded. Females with total litter loss were sacrificed on the day of litter loss, and the number of implantation sites was recorded; a sample of mammary tissue was examined and collected, and routine necropsy was performed. Females failing to litter were sacrificed on presumed gestation day 25; their uteri were evaluated for implantation sites and pregnancy status was confirmed by the Salewski staining technique. Histopathological examination was performed on abnormal tissues of dams.

b. **Fetuses and Offspring:** On GD 20, fetuses were dissected from the uterus and sexed; blood samples were obtained from the umbilical cord and the brains were removed and weighed. Fetuses were killed by chilling on a cool plate at approximately 0°C. Pups dying during lactation were examined grossly; culled pups were discarded without further examination. Pups treated through lactation day 21 were killed by carbon dioxide inhalation after blood sampling and subjected to gross necropsy. Brains were removed and weighed.

D. **DATA ANALYSIS**

1. **Statistical analyses:** Blood parameter and brain cholinesterase data were analyzed by parametric tests (Analysis of Variance) followed by Williams' test or nonparametric tests (Kruskal-Wallis) followed by Shirley's test. Where there were insufficient groups to support the use of the trend tests, these were replaced by Dunnett's test or the Wilcoxon test. Significance was reported at $p < 0.05$. Other parameters were not analyzed statistically.

2. **Indices:**

a. **Reproductive indices:** The following indices were calculated for maternal 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

The following index was calculated for dams allowed to litter:

Gestation index = (No. live litters/No. 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 postnatal 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

3. **Positive and historical control data:** Positive and historical control data were not required for this dose range-finding study.

II. RESULTS:

A. MATERNAL AND OFFSPRING OBSERVATIONS:

1a. Maternal mortality and clinical and functional observations:

All dams at 1250 mg/kg/day showed transient post-dosing salivation. Two dams died after 4 consecutive daily doses of 1250 mg/kg/day; one animal was killed after exhibiting body tremors and abnormal gait on GD 9 and another was found dead on GD 10. Due to these deaths, the dose was reduced to 1000 mg/kg/day. After decreasing the dose to 1000 mg/kg/day, body tremors, prostration, rales, prominent eyes, and/or a slow, abnormal gait were observed in standard arena observations during late gestation. Five animals were subsequently sacrificed between GD 16-19 due to these clinical signs. Dosing was then

suspended for this group (on GD 16-19) and all surviving animals were necropsied on GD 20. At 750 mg/kg/day, all dams showed transient post-dosing salivation. Two animals in this group were sacrificed, one on GD 16 after showing body tremors, abnormal gait, underactivity, and abnormal respiration; and another on GD 18 after showing a prostrate posture, body tremors, and prominent eyes. Dosing for this group was suspended by GD 18, and all surviving animals were necropsied on GD 20. Clinical signs were unremarkable at doses of 7.5 and 35 mg/kg/day. Transient post-dosing salivation was observed for 14/15 dams at 75 mg/kg/day and 15/15 dams at 150 mg/kg/day (as compared to 0/15 control dams). At 35 mg/kg/day, post-dosing salivation was restricted to single incidences in five dams in late gestation/early lactation. At 7.5 mg/kg/day, there were sporadic incidences of post dosing salivation. Arena observations at 7.5 mg/kg/day did not indicate any effect.

1b. Offspring mortality and clinical and functional observations:

Dosing of offspring (from dams treated at 7.5 mg/kg/day) at 450 mg/kg/day from PND 11 was associated with hypoactivity, pallor, and forelimb and head tremors. and being cold to touch on the second and third days of dosing. Pups showing severe clinical signs (11 of 35) were sacrificed for humane reasons. Additionally, 12 (of 35) pups at 450 mg/kg/day were found dead between PND 12-13, some without showing clinical signs prior to death. It is surprising that mortality occurred in the absence of cholinergic signs. This suggests the possibility that the test material exerted a mode of toxicity that is quite severe and yet perhaps independent of cholinesterase inhibition. Since these deaths were observed in offspring after the second and possibly the third doses, it suggests a more severe response after one or two doses than after a single dose. This dosage level was terminated at PND 11-13. Five (of 40) pups (also from dams treated at 7.5 mg/kg/day) treated with 200 mg/kg/day began to show body tremors between PND 12-14, and since it was anticipated that effects would continue to progress, this dosage was terminated at that time (i.e., on PND 12-15, 24 hours after the last dose). No treatment-related clinical signs were reported in juveniles treated with 7.5, 35, 75, or 150 mg/kg/day.

2. Maternal body weight and food consumption:

Selected group mean body weight and food consumption data for pregnant and nursing dams are summarized in Table 2 (Phase 1) and Table 3 (Phase 2). Body weight gains during gestation were slightly decreased in animals treated with 1250/1000 mg/kg/day. There were no body weight effects during gestation or lactation at 7.5, 35, 75, 150, or 750 mg/kg/day.

Food consumption during gestation was decreased in animals treated with 1250/1000 mg/kg/day. There were no food consumption effects during gestation at 7.5, 35, 75, 150, or 750 mg/kg/day.

Study Interval/Endpiont	0 mg/kg/day	7.5 mg/kg/day	750 mg/kg/day	1250/1000 mg/kg/day
TABLE 2. Selected maternal body weight and food consumption data during gestation and lactation for phase 1 animals				
Phase 1 Gestation (n = 8-15)				
Body wt. GD 0 (g)	241 ± 16	236 ± 17	237 ± 10	236 ± 12 (98)[a]
Body wt. GD 6 (g).	272 ± 22	268 ± 18	267 ± 12	266 ± 14 (98)
Body wt. GD 14 (g)	317 ± 32	311 ± 24	307 ± 14	299 ± 19 (94)
Body wt. GD 20 (g)	399 ± 45	387 ± 33	380 ± 19	367 ± 28 (92)
Wt. gain GD 0-6 (g)	31 ± 9	32 ± 4	30 ± 7	30 ± 7
Wt. gain GD 6-20 (g)	127 ± 26	119 ± 18	112 ± 9	101 ± 19 (80)
Food cons. GD 0-2 (g/rat/day)	27 ± 6	27 ± 3	27 ± 3	26 ± 2
Food cons. GD 6-9 (g/rat/day)	27 ± 4	27 ± 3	25 ± 2	23 ± 2 (85)
Food cons. GD 17-19 (g/rat/day)	27 ± 5	27 ± 5	27 ± 4	23 ± 6 (85)
Phase 1 Lactation (n = 8-10)				
Body wt. PND 1 (g)	308 ± 29	288 ± 20	–	–
Body wt. PND 11 (g)	346 ± 29	320 ± 19	–	–
Body wt. PND 21 (g)	325 ± 27	310 ± 25	–	–
Wt. gain PND 1–11 (g)	38 ± 11	32 ± 9	–	–
Wt. gain PND 1–21 (g)	17 ± 17	21 ± 10	–	–
Food cons. PND 1–3 (g/rat/day)	35 ± 5	36 ± 4	–	–
Food cons. PND 17–20 (g/rat/day)	72 ± 12	70 ± 9	–	-

Data taken from Tables 2A-7A, pp. 57, 59, 61, 63, 65, and 67, MRID 45627001.
a Number in parentheses is percent of control; calculated by reviewer.

Study Interval/Endpiont	0 mg/kg/day	7.5 mg/kg/day	35 mg/kg/day	75 mg/kg/day	150 mg/kg/day
TABLE 3. Selected maternal body weight and food consumption data during gestation and lactation for Phase 2 animals					
Phase 2 Gestation (n = 14-15)					
Body wt. GD 0 (g)	238 ± 7	235 ± 10	234 ± 11	235 ± 9	234 ± 7
Body wt. GD 6 (g)	281 ± 10	278 ± 13	274 ± 16	278 ± 13	274 ± 7
Body wt. GD 14 (g)	332 ± 15	327 ± 16	323 ± 24	329 ± 21	325 ± 12
Body wt. GD 20 (g)	414 ± 22	412 ± 21	406 ± 31	414 ± 26	411 ± 18
Wt. gain GD 0-6 (g)	43 ± 5	42 ± 5	40 ± 7	44 ± 7	40 ± 4
Wt. gain GD 6-20 (g)	133 ± 16	134 ± 12	132 ± 17	136 ± 16	137 ± 14
Food cons. GD 0-2 (g/rat/day)	30 ± 2	29 ± 3	29 ± 3	30 ± 3	29 ± 1
Food cons. GD 6-9 (g/rat/day)	29 ± 2	29 ± 2	29 ± 3	29 ± 3	29 ± 2
Food cons. GD 17-19 (g/rat/day)	32 ± 3	31 ± 3	30 ± 5	32 ± 3	32 ± 5
Phase 2 Lactation (n =10)					
Body wt. day 1 (g)	327 ± 22	321 ± 15	309 ± 30	321 ± 23	326 ±16
Body wt. day 11 (g)	367 ± 22	360 ± 15	346 ± 27	354 ± 22	365 ± 16
Body wt. day 21 (g)	351 ± 14	344 ± 14	341 ± 24	339 ± 26	349 ± 12
Wt. gain days 1-11 (g)	41 ± 9	39 ± 13	37 ± 11	33 ± 13	40 ± 9
Wt. gain days 1-21 (g)	25 ± 11	23 ± 9	32 ± 11	18 ± 11	24 ± 10
Food cons. days 1-3 (g/rat/day)	37 ± 6	39 ± 5	39 ± 4	38 ± 5	39 ± 5
Food cons. days 17-20 (g/rat/day)	80 ± 7	82 ± 7	86 ± 7	82 ± 9	81 ± 19

Data taken from Tables 2B-7B, pp. 58, 60, 62, 64, 66, and 68, MRID 45627001.

3. **Reproductive performance and litter observations:** The reproductive performance of animals killed on GD 20 is summarized in Table 4 (Phase 1) and Table 5 (Phase 2). No differences were observed at any dose between the treated and control groups for mean numbers of corpora lutea, implantations, live fetuses, resorptions, fetal body weights, fetal brain weights (not shown), or fetal sex ratios.

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TABLE 4. Reproductive performance of females killed on GD 20 - Phase 1				
Endpoint	0 mg/kg/day	7.5 mg/kg/day	750 mg/kg/day	1250/1000 mg/kg/day
No. dams	5	5	13	8
No. with live young	5	5	13	8
Corpora lutea/dam	17.2 ± 2.3	15.8 ± 1.5	15.5 ± 2.8	15.4 ± 1.7
Implantations/dam	15.2 ± 1.9	14.8 ± 1.9	13.7 ± 1.5	15.1 ± 1.6
Live fetuses/dam	14.0 ± 3.1	14.0 ± 2.7	13.2 ± 1.8	14.6 ± 1.6
Total resorptions/dam	1.2	0.8	0.5	0.5
Pre-implantation loss (%)	11.5	6.5	10.2	2.2
Post-implantation loss (%)	8.4	5.9	4.0	3.2
Fetal body wt.	3.81 ± 0.25	3.82 ± 0.31	3.97 ± 0.33	3.90 ± 0.23
Sex ratio (% male)	41.3	46.1	44.6	48.1

Data taken from Tables 9A and 10A, pp. 71 and 73, MRID 45627001.

TABLE 5. Reproductive performance of females killed on GD 20 - Phase 2					
Endpoint	0 mg/kg/day	7.5 mg/kg/day	35 mg/kg/day	75 mg/kg/day	150 mg/kg/day
No. dams	5	5	5	5	5
No. with live young	5	5	5	5	5
Corpora lutea/dam	16.2 ± 2.4	15.6 ± 1.1	15.8 ± 0.8	16.6 ± 1.5	15.2 ± 1.3
Implantations/dam	15.2 ± 1.9	15.4 ± 1.1	15.0 ± 0.7	16.0 ± 0.7	15.2 ± 0.8
Live fetuses/dam	14.0 ± 2.4	14.6 ± 1.8	14.0 ± 0.7	15.2 ± 0.8	14.4 ± 1.7
Total resorptions/dam	1.2 ± 1.1	0.8 ± 0.9	1.0 ± 1.0	0.8 ± 0.9	0.8 ± 0.8
Pre-implantation loss (%)	5.7	3.8	4.9	4.4	1.3
Post-implantation loss (%)	8.3	5.4	6.5	4.9	5.5
Fetal body wt.	4.05 ± 0.30	4.04 ± 0.21	4.17 ± 0.14	4.14 ± 0.21	4.16 ± 0.22
Sex ratio (% male)	46.4	55.0	53.4	51.6	40.2

Data taken from Tables 9B and 10B, pp. 72 and 74, MRID 45627001.

Reproductive and litter data for dams allowed to litter and rear their young are given in Table 6 (Phase 1) and Table 7 (Phase 2). There was no evidence of an effect at doses as high as 1250/1000 mg/kg/day on parameters of reproductive performance among dams allowed to litter. No differences were observed between the treated and control groups for pregnancy rate, gestation length, mean numbers of implantations, total litter size, pup sex ratios, pup body weight, and pup survival.

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TABLE 6. Reproductive performance of females allowed to litter - Phase 1		
Endpoint	0 mg/kg/day	7.5 mg/kg/day
No. Females	10	10
No. pregnant	10	10
Gestation length (days)	22.05	22.25
No. live litters	10	10
Gestation index (%)	100	100
No. with live at weaning	10	10
Total litter loss	0	0
Implants/dam	15.7 ± 2.2	14.5 ± 1.1
Total litter size (PND 1)	14.9 ± 2.2	13.4 ± 1.1
Live litter size PND 1 PND 4 (precull) PND 11	14.9 ± 2.2 14.8 ± 2.1 9.6 ± 0.7	13.4 ± 1.1 13.3 ± 1.2 9.9 ± 0.3
Live Birth Index (%)	100	100
Viability Index (%)	99.4	99.2
Lactation Index PND 11 (%)	96.0	99.0
Sex ratio at birth (% male)	48.9 ± 13.2	44.3 ± 9.6
Pup body wt. - male [a] PND 1 PND 11 PND 21	6.7 ± 0.4 24.4 ± 2.3 54.3 ± 4.8	6.7 ± 0.5 23.9 ± 3.1 51.9 ± 6.7
Pup wt. change - male [a] PND 1-11 PND 11-21	17.8 ± 2.2 29.9 ± 2.9	17.1 ± 2.9 27.7 ± 4.7
Pup body wt. - female [a] PND 1 PND 11 PND 21	6.3 ± 0.5 23.4 ± 2.8 52.2 ± 5.9	6.4 ± 0.4 22.7 ± 2.9 49.5 ± 6.4
Pup wt. change - female [a] PND 1-11 PND 11-21	17.2 ± 2.5 28.8 ± 3.4	16.3 ± 2.7 26.8 ± 4.0

Data taken from Tables 8A, 12A, 13A, 14A, 19A, 20A, 23A, and 24A, pp. 69, 77, 79, 81, 91, 93, 99, and 101, respectively. MRID 45627001.

a Includes treated pups only (n=15-20 for males, 14-22 for females).

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TABLE 7. Reproductive performance of females allowed to litter - Phase 2				
Endpoint	0 mg/kg/day	7.5 mg/kg/day	35 mg/kg/day	75 mg/kg/day	150 mg/kg/day
No. Females	10	10	10	10	10
No. pregnant	10	10	10	10	10
Gestation length (days)	22.25	22.3	22.05	22.45	22.15
No. live litters	10	10	10	10	10
Gestation index (%)	100	100	100	100	100
No. with live at weaning	10	10	10	10	10
Total litter loss	0	0	0	0	0
Implants/dam	15.2 ± 1.9	15.4 ± 1.1	15.0 ± 0.7	16.0 ± 0.7	15.2 ± 0.8
Total litter size (PND 1)	14.0 ± 2.4	14.6 ± 1.8	14.0 ± 0.7	15.2 ± 0.8	14.4 ± 1.7
Live litter size PND 1 PND 4 (precull) PND 11	12.5 ± 2.6 12.5 ± 2.6 9.5 ± 1.0	13.0 ± 1.7 12.8 ± 1.8 7.9 ± 0.3	14.1 ± 1.3 14.0 ± 1.4 8.0 ± 0.0	12.8 ± 2.8 12.8 ± 2.8 7.9 ± 0.3	12.9 ± 1.8 12.9 ± 1.8 8.0 ± 0.0
Live Birth Index (%)	100	99.2	100	100	100
Viability Index (%)	100	99.3	99.2	100	100
Lactation Index PND 11 (%)	99.0	98.8	100	100	100
Sex ratio at birth (% male)	49.2 ± 16.7	50.3 ± 14.0	49.0 ± 8.4	48.5 ± 14.9	45.8 ± 18.9
Pup body wt. - male [a] PND 1 PND 11 PND 21	6.8 ± 0.8 25.0 ± 2.8 53.2 ± 5.0	6.6 ± 0.3 24.8 ± 1.7 51.8 ± 3.9	6.5 ± 0.6 25.8 ± 1.2 53.2 ± 3.0	6.8 ± 1.0 25.7 ± 3.4 54.2 ± 7.0	6.7 ± 0.8 27.7 ± 2.0 56.8 ± 3.9
Pup wt. change - male [a] PND 1-11 PND 11-21	18.2 ± 2.3 27.8 ± 2.8	18.2 ± 1.7 26.9 ± 2.5	19.3 ± 1.1 27.5 ± 2.2	18.9 ± 2.8 28.5 ± 4.5	21.0 ± 1.7 29.1 ± 2.5
Pup body wt. - female [a] PND 1 PND 11 PND 21	6.4 ± 0.7 23.8 ± 2.7 50.3 ± 4.5	6.4 ± 0.4 24.9 ± 1.3 51.5 ± 2.8	6.2 ± 0.5 24.5 ± 1.6 50.3 ± 3.3	6.4 ± 0.9 24.8 ± 2.7 52.5 ± 5.2	6.5 ± 1.0 26.4 ± 2.9 53.8 ± 5.9
Pup wt. change - female [a] PND 1-11 PN 11-21	17.5 ± 2.3 26.5 ± 2.7	18.5 ± 1.3 26.6 ± 2.0	18.3 ± 1.5 25.8 ± 2.2	18.4 ± 2.2 27.3 ± 3.8	19.9 ± 2.5 27.4 ± 3.7

Data taken from Tables 8B, 9B, 12B, 13B, 14B, 19B, 20B, 23B, and 24B, pp. 70, 72, 78, 80, 82, 92, 94, 100, and 102, respectively. MRID 45627001.

a Includes treated pups only (n=18-21 for males, 19-28 for females).

4. **Postmortem results:** No treatment-related gross lesions were observed in dams killed on GD 20 or in dams and pups killed on lactation day 21. Brain weight data for 21-day old pups are given in Table 8 (Phase 1) and Table 9 (Phase 2). No differences in brain weight were found among treatment groups.

TABLE 8. Absolute and relative brain weights of 21-day old pups - Phase 1		
Weight	0 mg/kg/day	7.5 mg/kg/day
Male (n=20-21)		
Body weight (g)	54 ± 4.7	51 ± 6.6
Absolute brain wt. (g)	1.458 ± 0.044	1.464 ± 0.059
Relative brain wt. (% body wt.)	2.741 ± 0.233	2.901 ± 0.324
Female (n=19-22)		
Body weight (g)	51 ± 6.0	49 ± 6.2
Absolute brain wt. (g)	1.441 ± 0.039	1.410 ± 0.078
Relative brain wt. (% body wt.)	2.836 ± 0.344	2.931 ± 0.321

Data taken from Table 27A, p. 107, MRID 45627001.

TABLE 9. Absolute and relative brain weights of 21-day old pups - Phase 2					
Weight	0 mg/kg/day	7.5 mg/kg/day	35 mg/kg/day	75 mg/kg/day	150 mg/kg/day
Male (n=18-22)					
Body weight (g)	53 ± 4.8	51 ± 4.0	52 ± 2.9	53 ± 6.9	55 ± 3.5
Absolute brain wt. (g)	1.475 ± 0.089	1.462 ± 0.055	1.482 ± 0.059	1.482 ± 0.074	1.513 ± 0.050
Relative brain wt. (% body wt.)	2.820 ± 0.195	2.901 ± 0.260	2.856 ± 0.146	2.852 ± 0.319	2.748 ± 0.175
Female (n=17-21)					
Body weight (g)	50 ± 4.5	50 ± 2.7	49 ± 3.3	51 ± 4.9	52 ± 5.5
Absolute brain wt. (g)	1.442 ± 0.053	1.437 ± 0.077	1.443 ± 0.062	1.450 ± 0.039	1.429 ± 0.043
Relative brain wt. (% body wt.)	2.928 ± 0.259	2.866 ± 0.223	2.938 ± 0.137	2.882 ± 0.270	2.757 ± 0.246

Data taken from Table 27B, p. 108, MRID 45627001.

B. **CHOLINESTERASE ACTIVITY:** Plasma, RBC, and brain cholinesterase activity levels for dams and offspring are summarized in Table 10 (Phase 1) and Table 11 (Phase 2). At 1250/1000 mg/kg/day, there was substantial inhibition in all compartments (39% plasma, 80% RBC, 35% brain) for adults, a decrease in RBC cholinesterase (27%) for male and female fetuses, and a decrease in brain cholinesterase for female fetuses (30%. At 750 mg/kg/day, adults (GD 20) showed decreased plasma (28%), RBC (78%), and brain (49%) cholinesterase activities and fetal RBC cholinesterase activity was also decreased 27% as compared with controls. GD 20 dams treated with 150 mg/kg/day exhibited decreased

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plasma (18%) and RBC (59%) cholinesterase activities, and GD 20 dams treated with 75 mg/kg/day also had decreased plasma (13%) and RBC (33%) cholinesterase activities. GD 20 adult brain cholinesterase and fetal plasma, RBC, and brain cholinesterase activities were not affected at 75 or 150 mg/kg/day. Plasma, RBC, and brain cholinesterase activities were decreased 22%, 40%, and 20%, respectively, in pups dosed fro 3 days at 200 mg/kg/day compared to Phase 2 juvenile controls. In the PND 21 offspring at 75 and 150 mg/kg/day, plasma, RBC, and brain cholinesterase activities were decreased compared to controls. Decreases were 11%, 42%, and 9% for males and 16%, 41%, and 7% for females at 75 mg/kg/day; and 22%, 58%, and 18% for males and 26%, 65%, and 19% for females at 150 mg/kg/day, for plasma, RBC, and brain compartments, respectively. For PND 21 offspring at 7.5 and 35 mg/kg/day, only RBC cholinesterase activity was decreased (19% and 30% for males and 12% and 26% for females, respectively) compared to controls. Since inhibition of RBC cholinesterase was dose-related and statistically significant across all doses and in both sexes, findings at all doses are considered to be biologically relevant. It is additionally noted that these findings are consistent with RBC cholinesterase inhibition observed in the comparative cholinesterase study in rats with malathion (MRID 45566201), in which RBC cholinesterase was inhibited 17% in PND 21 males and 15% in PND 21 females at 5 mg/kg/day following the same exposure protocol. A NOAEL for RBC cholinesterase inhibition was not identified in either study.

TABLE 10. Cholinesterase Activities - Phase 1				
Tissue	0 mg/kg/day	7.5 mg/kg/day	750 mg/kg/day	1250/1000 mg/kg/day
Gestation Day 20				
Dams (n=5)	(n=5)	(n=5)	(n=13) a	(n=9) a
Plasma (U/L)	1339 ± 108.7	1349 ± 256.4	962** ± 227.2 (28) b	820** ± 286.8 (39)
RBC (U/L)	1145 ± 108.1	1035 ± 102.5 (10)	252** ± 83.8 (78)	231** ± 113.7 (80)
Brain (U/kg)	12980 ± 391.5	13050 ± 367.4	6658** ± 2091.6 (49)	8378** ± 2165.5 (35)
Fetuses				
Plasma (U/L) ♂ & ♀ combined	256 ± 33.4	247 ± 26.5 (4)	240 ± 511.2 (6)	238 ± 9.9 (7)
RBC (U/L) ♂ & ♀ combined	765 ± 22.4	640 ± 100.9 (16)	555* ± 152.5 (27)	558* ± 80.4 (27)
Brain (U/kg) Male Female	1200 ± 223.6 1420 ± 175.4	1170 ± 83.7 (3) 1240 ± 219.1 (13)	1260 ± 339.9 (+5) 1470 ± 309.4 (+4)	1238 ± 209.7 (+3) 988 ± 188.7 (30)
Tissue	**0 mg/kg/day**	**7.5 mg/kg/day**		
PND 21				
Male pups (n=20-21)				
Plasma (U/L)	520 ± 87.5	527 ± 49.9 (+1)		
RBC (U/L)	1564 ± 190.5	1464 ± 420.4 (6)		
Brain (U/kg)	10783 ± 564.6	10648 ± 442.9 (1)		
Female pups (n=19-22)				
Plasma (U/L)	493 ± 53.7	511 ± 33.3 (+4)		
RBC (U/L)	1613 ± 177.4	1498 ± 221.9 (7)		
Brain (U/kg)	10713 ± 521.2	10443 ± 513.9 (3)		
Tissue	**0 mg/kg/day**	**200 mg/kg/day**		
	PND 15	**PND 12-15**		
Male pups	(n=10) c	(n=19) d		
Plasma (U/L)	736 ± 57	578*** ± 55.7 (22)		
RBC (U/L)	1488 ± 144.9	893*** ± 223.6 (40)		
Brain (U/kg)	8345 ± 431.1	6708*** ± 563.3 (20)		
Female pups	(n=9) c	(n=20) d		
Plasma (U/L)	707 ± 57.6	542*** ± 46.1 (23)		
RBC (U/L)	1450 ± 176.8	878*** ± 185.3 (39)		
Brain (U/kg)	8394 ± 396.4	6220*** ± 732.8 (36)		

Data taken from Tables 28A, 29A, 30, 32A, 33A, pp. 109, 111, 113-115, & 117. MRID 45627001.

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*p<0.05; **p<0.01; ***p<0.001.
a Dosing terminated on GD 16-19; sacrificed on GD 20.
b Number in parentheses is % inhibition compared to control.
c Phase 2 pups, originating from control dams.
d Phase 1 pups originating from 7.5 mg/kg/day dams; dosing terminated on PND 11-14, sacrificed 1 day later.

TABLE 11. Cholinesterase Activities - Phase 2				
Tissue	0 mg/kg/day	7.5 mg/kg/day	35 mg/kg/day	75 mg/kg/day	150 mg/kg/day
Gestation Day 20					
Dams (n=5)					
Plasma (U/L)	1301 ± 118.9	1462 ± 229.6 (+12) a	1304 ± 261.2 (0)	1137 ± 195.2 (13)	1071 ± 177.2 (18)
RBC (U/L)	1210 ± 115.4	1475 ± 386.9 (+22)	1095 ± 321.3 (10)	815* ± 129.4 (33)	495** ± 77.9 (59)
Brain (U/kg)	13100 ± 430.1	13180 ±290.7 (+1)	13330 ± 216.8 (+2)	12890 ± 198.1 (2)	12730 ± 667.6 (3)
Fetuses					
Plasma (U/L) (♂ & ♀combined)	268 ± 16.9	260 ± 14.5 (3)	243* ± 16.2 (9)	238** ± 13 (11)	240** ± 14.6 (10)
RBC (U/L) (♂ & ♀ combined)	900 ± 58.6	965 ± 82.2 (+7)	940 ± 125.7 (+4)	835 ± 48.7 (7)	830 ± 59.7 (8)
Brain (U/kg) Male Female	1400 ± 145.8 1610 ± 263.2	1590 ± 129.4 (+14) 1600 ± 61.2 (1)	1520 ± 103.7 (+9) 1390 ± 108.4 (14)	1773 ± 507.4 (+27) 1720 ± 251.5 (+7)	1620 ± 83.7 (+16) 1460 ± 102.5 (9)
PND 21					
Male pups (n=19-20)					
Plasma (U/L)	516 ± 61.9	533 ± 62.2 (+3)	498 ± 44.2 (3)	460 ± 47.5 (11)	405 ± 41.2 (22)
RBC (U/L)	1631 ± 258.3	1318** ±166.8 (19)	1142** ± 184.3 (30)	948** ± 221.2 (42)	691** ± 161.6 (58)
Brain (U/kg)	10753 ± 604	10455 ± 322.4 (3)	10380 ± 379.5 (3)	9780** ± 674.2 (9)	8779** ± 644.7 (18)
Female pups (n=18-20)					
Plasma (U/L)	516 ± 67.9	503 ± 63.2 (3)	493 ± 65.4 (4)	431** ± 59.1 (16)	380** ± 67.8 (26)
RBC (U/L)	1550 ± 197.8	1359* ± 296.8 (12)	1153** ± 254.4 (26)	922** ± 155.2 (41)	540** ± 111.6 (65)
Brain (U/kg)	10561 ± 353.4	10470 ± 324.2 (1)	10370 ± 382 (2)	9837** ± 653.2 (7)	8528**± 882.2 (19)

Data taken from Tables 28B, 29B, 32B, 33B, pp. 110, 112, 116, & 118, MRID 45627001.
*p<0.05; **p<0.01.
a Number in parentheses is % inhibition compared to control.

C. BLOOD CHEMISTRY:

No significant treatment-related effects were noted for blood sodium, potassium, bicarbonate, chloride, calcium or magnesium for Phase 2 dams at GD 20 or Phase 2 dosed offspring at PND 21 (data not presented in DER).

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III. DISCUSSION:

.This group of studies was conducted to select doses for a developmental neurotoxicity study with malathion.

Two dams died after 4 consecutive daily doses of 1250 mg/kg/day. After decreasing the dose to 1000 mg/kg/day, body tremors, prostration, rales, prominent eyes, and a slow, abnormal gait were observed during late gestation, necessitating premature sarifice of 5 dams during late gestation. Dosing was suspended during GD 16-19. At 750 mg/kg/day, all dams showed transient post-dosing salivation, and body tremors, abnormal gait, hypoactivity, and abnormal respiration during GD 16-18, necessitating premature sacrifice of 2 dams during gestation prior to suspending dosing for the group on GD 18. Clinical signs were unremarkable in dams at doses of 7.5, 35, 75, and 150 mg/kg/day. Body weight gains and food consumption during gestation were slightly decreased in dams treated with 1250/1000 mg/kg/day. There were no body weight effects during gestation or lactation at 7.5, 35, 75, 150, or 750 mg/kg/day.

For dams that were sacrificed on GD 20, there were no differences between the treated and control groups in mean numbers of corpora lutea, implantations, live fetuses, resorptions, fetal body weights, fetal brain weights, or fetal sex ratios, even at doses of 750 and 1250/1000 mg/kg/day, where there were rather severe clinical signs of toxicity. For dams allowed to litter (at 7.5, 35, 75, and 150 mg/kg/day), no differences were observed between the treated and control groups for pregnancy rate, mean numbers of implantations, total litter size, or pup sex ratios.

Severe toxicity was observed following direct gavage dosing to pups (starting at PND 11) at 200 and 450 mg/kg/day. Twelve of 35 offspring treated at 450 mg/kg/day were found dead between PND 12-14, some without showing clinical signs prior to death. Pups in this group also showed hypoactivity, pallor, forelimb and head tremors, and were cold to touch on the second and third days of dosing; 11 of 35 pups were sacrificed for humane reasons. Therefore, the total number of pup mortalities was 23/35, and the 450 mg/kg/day group was terminated at PND 11-13. Juveniles treated with 200 mg/kg/day began to show body tremors between PND 12-14 and were terminated at that time (i.e., PND 12-15, 24 hours after the last dose). No treatment-related clinical signs were reported in juveniles treated by direct gavage dosing with 7.5, 35, 75, or 150 mg/kg/day from PND 11-21.

No treatment-related gross lesions were observed in dams killed on GD 20 or in dams and pups killed on lactation day 21. Brain weights for fetuses and 21-day old pups were not affected by treatment.

At gestation day 20 (following gavage dosing from GD 6-20), RBC cholinesterase was inhibited in dams at 75 mg/kg/day (33%) and above (i.e., 59% at 150 mg/kg/day, 78% at 750 mg/kg/day, and 80% at 1250/1000 mg/kg/day). For GD 20 dams at 750 mg/kg/day, plasma and brain cholinesterase were inhibited 28 and 49%, respectively; at 1250/1000 mg/kg/day, plasma and brain cholinesterase were inhibited 39 and 35%, respectively. (For both of these groups, dosing had been discontinued between GD 16-19.) In fetuses, RBC cholinesterase

was inhibited at 750 mg/kg/day (27%) and at 1250/1000 mg/kg/day (27%), but plasma and brain cholinesterase were not affected by treatment at any dose level.

In PND 21 pups that had received direct gavage dosing of malathion from PND 11-21, plasma cholinesterase was inhibited 22-26% at 150 mg/kg/day, and brain cholinesterase was inhibited at doses of 75 mg/kg/day (7-9%) and 150 mg/kg/day (18-19%). RBC cholinesterase was inhibited in a dose dependant manner at 7.5 mg/kg/day and above for Phase 2 PND 21 males (19%) and females (12%). Phase 1 PND 21 male and female pups did not demonstrate RBC cholinesterase inhibition at 7.5 mg/kg/day. Nevertheless, the RBC finding at 7.5 mg/kg/day is consistent with findings in the comparative cholinesterase study with malathion (MRID 45566201).

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

In offspring, overt clinical signs of toxicity (body tremors and moribundity) were observed at a dose of 200 mg/kg/day and above. RBC cholinesterase inhibition was observed at all doses tested (i.e., 7.5 mg/kg/day and above) in PND 21 pups. Brain cholinesterase inhibition was seen at 75 mg/kg/day and above, and plasma cholinesterase was inhibited at 150 mg/kg/day and above. For GD 20 fetuses, RBC cholinesterase was inhibited at 750 mg/kg/day and above.

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

This study is classified **Acceptable/Nonguideline** as a dose range-finding study and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). (Note: this conclusion represents the opinion of one of the authors of this DER, and was supported by peer review of the Hazard Identification Assessment Review Committee [TXR 0050804] and the Developmental Neurotoxicity Protocol Review Committee [TXR 0051035]. A dissenting opinion, from the other DER author, is presented in Appendix 1 to this DER.)

IV.   STUDY DEFICIENCIES:

No information was provided to justify the timing of sample collection for cholinesterase measures (i.e., termination of dams and fetuses at 3 hours post-dosing, and PND 21 pups at 2 hours post-dosing).

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Appendix 1 - Dissenting Opinion to Adequacy of Dose Levels

MEMORANDUM

From: Brian Dementi
Date: 08/13/02 03:52 PM
To: Susan Makris/DC/USEPA/US@EPA
cc: Elizabeth Doyle/DC/USEPA/US@EPA
Subject: Secondary Review of Range-Finding Malathion DNT Study

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Susan,

Please find appended my comments directed to the draft range-finding DNT study on malathion. I anticipate that much of this may wind up as a dissenting opinion rather than editorial changes that will be honored. but it remains to be seen. The bottom line is that the range-finding study is inadequate to the objective of providing information for dose selection in the definitive DNT study. Also it did not ferret out the magnitude of offspring versus adult susceptibility in terms of acute cholinergic toxicity, nor explain or otherwise address the surprising level of toxicity among dams heretofore not seen in Guideline studies.

I have copies of the referenced studies and memoranda referred to in my office.

Best Wishes,
Brian D.
Attachment: RFDNTREV.wpd

Range-Finding DNT Study Review. Suggested revisions to the draft DER are rendered in bold italics.

1) (p. 2) Concerning the classification **Acceptable/Nonguideline,** I find problematic the study's acceptability for range-finding purposes. The most specific reason is the inadequacy of the dose range employed to estimate the MTD for dams to be used in the definitive study.

As explained in my appended Email of 6/20/02 to Marcia Mulkey, in the preliminary range-finding study, severe toxicity was observed in dams at the lowest dose tested of 750 mg/kg/day and similarly in offspring that of 200 mg/kg/day. With this information in mind, the high dose level selected for this final range-finding study was but 150 mg/kg/day. a dose level targeted to be an MTD in offspring. but clearly below the anticipated MTD for dams. There was no attempt to identify an MTD for dams to be used in this defining range-finding study under review. Such data is needful not only to be predictive of an MTD for dams for use in the definitive DNT study, but to clarify the magnitude of comparative susceptibility of offspring versus adult in terms of clinical cholinergic toxicity. In other words. given the evidence of enhanced acute toxicity in offspring (200 mg/kg/day) versus that in adults (750 mg/kg/day) as identified in the preliminary studies. there is an incumbency under FQPA to *quantitate* with more precision the magnitude of that disparity. The existing preliminary data suggests an offspring susceptibility factor on the

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order of 750 mg/kg/day/200 mg/kg/day, or 3.75-fold. This evidenced of enhanced offspring susceptibility rendered in terms of acute cholinergic toxicity, imprecise though it may be as it stands, serves to augment the evidence of susceptibility at lower doses as observed in the definitive DNT and cholinesterase studies.

In essence, the study is not acceptable as a range-finding study because it did not identify, or approximate, an MTD in dams for use in the definitive Guideline DNT study.

2) (p. 3) Under "Study schedule", concerning the sentence which reads "Controls and rats in the 7.5 mg/kg/day groups *from the same phase 1 study* were treated as scheduled." *It should be noted that in addition, offspring (2 males and 2 females per litter) from the control and 7.5 mg/kg/day groups were tested at 200 and 450 mg/kg, respectively. This was considered necessary as an effort to salvage information from phase 1 after 1250, 1000 and 750 mg/kg/day in dams proved to be excessively toxic to dams, requiring suspension of dosing in this higher dosage range. The 200 and 450 mg/kg/day dosage levels proved to be excessively toxic to offspring, also necessitating the suspension of dosing in these offspring.* [EDITORIAL NOTE: THIS LANGUAGE HAS BEEN ADDED TO THE DER TEXT.]

3) (p. 5) Under item 5, some rationale is needed for dose selection, yet we may not fully embrace what rationale the registrant used.

4) (p. 9) Under item 1b, there is the sentence: "Additionally, 12 (of 35) pups at 450 mg/kg/day were found dead between PND 12-13, some without showing clinical signs prior to death." *It is surprising that mortality occurred in the absence of cholinergic signs. This suggests that the test material exerted a mode of toxicity that is quite severe and yet possibly independent of cholinesterase inhibition, or that the test material was of altered composition. The test material exhibited an enhanced order of toxicity in this study as compared with other Guideline studies, that has not been explained. Since these deaths were observed in offspring after the second and possibly the third doses, it suggests a more severe response after one or two doses than after one dose.* [EDITORIAL NOTE: THIS LANGUAGE HAS BEEN ADDED, IN PART, TO THE DER TEXT.]

5) (p. 9) Under item 2, it is explained that among dams there were no body weight or food consumption effects at dosage levels as high as 750 mg/kg/day. *There was therefore no evidence based upon body weight or food consumption of an MTD having been achieved among dams at doses as high as 750 mg/kg/day, let alone 150 mg/kg/day.*

6) (p. 11) Under item 3: *There was no evidence of an effect at doses as high 1250/1000 mg/kg/day on parameters of reproductive performance among dams allowed to litter.* [EDITORIAL NOTE: THIS LANGUAGE HAS BEEN ADDED TO THE DER TEXT.]

7) (p. 15) "At 750 mg/kg/day, adults (GD 20) showed decreased ....compared with controls." *These findings are possibly indicative of an MTD among dams, but in the absence of data between the 750 mg/kg/day and 7.5 mg/kg/day dose levels in phase 1, the findings would not support 150 mg/kg/day as an MTD for dams.*

8) (p. 15-16) "GD 20 dams treated with 150 mg/kg/day exhibited decreased plasma (18%) and RBC (59%) cholinesterase activities. and GD 20 dams treated with 75 mg/kg/day also had decreased plasma (13%) and RBC (33%) cholinesterase activities. GD 20 adult brain cholinesterase and fetal plasma. RBC. and brain cholinesterase activities were not affected at 75 or 150 mg/kg/day (emphasis added)." *These observations serve to further illustrate and substantiate the fact that 150 mg/kg/day is not an MTD in dams, and that dosing of dams during gestation at this level provides an inadequate challenge to the developing individual in utero. In other words, dosing pregnant dams at levels no higher than 150 mg/kg/day would not be expected to provide an adequate testing procedure to exploit the full potential for the assessment of developmental anomalies.*

9) Under the Discussion Section (p. 19), suggested revisions to the following statements as quoted from the text are given below.

"After decreasing the dose to 1000 mg/kg/day..........were observed during late gestation, *necessitating premature sacrifice of 5 dams during late gestation. Dosing was suspended during GD 16-19."* [EDITORIAL NOTE: THIS LANGUAGE HAS BEEN ADDED TO THE DER TEXT.]

"At 750 mg/kg/day. all dams showed transient post-dosing salivation........during GD 16-18, *necessitating premature sacrifice of 2 dams during gestation prior to suspending dosing for the group on GD 18."* [EDITORIAL NOTE: THIS LANGUAGE HAS BEEN ADDED TO THE DER TEXT.]

"There were no body weight effects during gestation or lactation at 7.7, 35, 75, 150, or 750 mg/kg/day. *indicating the absence of an MTD, certainly at 150 mg/kg/day."*

"Twelve of 35 offspring treated at 450 mg/kg/day were found dead between PND 12-14. *some without showing clinical signs prior to death."* [EDITORIAL NOTE: THIS LANGUAGE HAS BEEN ADDED TO THE DER TEXT.]

"In fetuses. RBC cholinesterase was inhibited at 750 mg/kg/day (27%) and at 1250/1000 mg/kg/day (27%). but ....at any dose level. *In Phase 2, RBC cholinesterase was not inhibited in fetuses at the highest dose tested, 150 mg/kg/day. This finding does not support the 150 mg/kg/day dose level in dams as affording an adequate challenge to the developing individual in utero, suggesting the need for selecting a dose level for dams in the definitive study that would exceed 150 mg/kg/day, which the other range-finding data in dams would indicate to be entirely possible somewhere not far below 750 mg/kg/day, which was evidently too toxic for dams."*

10) Under the Study Deficiencies section. the following additional text beyond the paragraph found there is suggested:

There is no evidence among the many parameters in this study to conclude that 150 mg/kg/day could be expected to serve as an MTD, or otherwise to be characterized as an acceptable high dose level, for dams as required for the Guideline DNT study. Furthermore, there is inadequate evidence in this range-finding study to support a conclusion that the developing individual would be influenced at this dose level (150 mg/kg/day) as administered to dams.

One must conclude therefore, that insofar as the findings in this range-finding data would serve to indicate dose selections for dams in the definitive DNT study, dose levels higher than 150 mg/kg/day would be necessary. Since inexplicably severe dam toxicity was observed at 750 mg/kg/day, and no further testing was conducted on dams at dose levels above 150 mg/kg/day, but below 750 mg/kg/day, one must conclude that a deficiency resides with the fact that further testing within this range was not pursued in order to reach the desired objective of identifying an adequate high dose (and lower dose levels as well) for the conduct of an acceptable Guideline DNT study. Furthermore, there should have been some explanation for the surprising degree of toxicity as observed in dams at and above the 750 mg/kg/day level, and to assess at what lower levels this surprising toxicity might extend. Additional study is also indicated to quantitate the magnitude of offspring versus adult susceptibility in terms of acute cholinergic toxicity.

The intended objective of the definitive testing is to conduct __both__ a satisfactory Guideline DNT study, as well as a satisfactory assessment of the consequences of direct dosing of offspring during PND 11-21. The latter aspect of this testing is not covered under the Guideline DNT protocol. The dose range-finding studies indicate that offspring are more remarkably affected than adults, 200 mg/kg/day in offspring, versus 750 mg/kg/day in dams. It is obvious that the conduct of both phases of this study are in conflict in terms of dosing requirements for both dams and offspring. Dose levels above 150 mg/kg/day which are identified as needed for a definitive DNT study are expected to be intolerable to offspring. However, the need for lower dose testing in offspring should not preclude adequate dosing for a definitive DNT study. The data indicate that a dose range likely to be somewhat below 750 mg/kg/day would be necessary for dosing of dams. The study is deficient in not determining that dose range, i.e. this range-finding study does not provide the guidance needed for the performance of an Acceptable Guideline DNT Study.

I would like to conclude these comments with the following historical record concerning the MTD assessment:

In her Email of March 13, 2001, Ms Carmelita White advised myself and others of the content of a conversation she had the same day with the registrant's representative concerning the initiation of the definitive DNT study at dose levels of 150, 50 and 5 mg (interpreted as meaning mg/kg/day). Ms White appeared to be affirming in this Email an earlier agreement with the registrant to the effect that the study should be "....based only upon the MTD for the pups....." I should note that the term *MTD* was employed in her letter. Ms White says in her closing sentence: "If you have any comments regarding the dosing, please respond immediately so that we may discuss any recommendations to Cheminova." In other words, the Email appears to be advising that the study was to be initiated at that moment, and was seeking timely input from the addressees concerning the adequacy of the dose level selection.

As the reviewer of the earlier submitted 6(a)(2) submissions on the range-finding DNT and cholinesterase studies [TXR014413 (12/14/00); TXR014544 (4/19/01)]. I should advise that concerns had been expressed over the adequacy of dosing which led to the conclusion that 150 mg/kg/day would not be adequate as a high test dose for a definitive DNT study as conducted by Guideline testing procedures. In view of this, I responded to Ms White the same day (3/13/01)

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conveying this concern to her. While citing the December 14, 2000 Toxicology Branch review of the range-finding 6(a)(2) data submitted by Cheminova July 10, 2000, I advised "So this data in essence supports the previous data showing that 150 mg/kg/day would not be a sufficiently high dose for the definitive DNT study." I also expressed concern that the December 14 review had not been forwarded to the registrant as HED's response to the earlier 6(a)(2) submission. Much of this concern as to dose selection is presented in that 12/14/00 review.

At a later date, Ms white forwarded to myself and others via her Email of April 19, 2001, correspondence dated April 16 from the registrant's representative, specifically Ms Diane Allemang, in which Ms Allemang says in her opening sentence: "In response to your request during a telephone call with Paul Whatling of Jellinek, Schwartz & Connolly, Inc., on March 14, 2001, Cheminova A/S (Cheminova), is providing the Agency with the following justification for the dose level that Cheminova selected for the developmental neurotoxicity (DNT) study for malathion that was required in EPA's September 10, 1999, data call-in notice." Ms Allemang then proceeds to justify the dose selection of 0, 5, 50 and 150 mg/kg/day for the definitive DNT study. One should note that while an opportunity apparently existed as late as March 13 to set dose levels for the definitive DNT study, such opportunity had passed by the time of receipt of the April 16 letter from Ms Allemang, as by then Cheminova had made its decision and initiated the study.

Upon receipt of Ms Allemang's April 16 letter, I drafted a response thereto as communicated via my Email of June 13, 2001 to Carmelita White, in which I questioned Ms Allemang's rationale for dose selection for the definitive DNT study, in essence reiterating the views expressed previously concerning the inappropriate dose selection for the definitive DNT study.

In her April 16 letter, Ms Allemang says, among other things, "In the first range-finding study, 200 mg/kg/day malathion directly dosed to pups resulted in significant clinical signs (body tremors) and premature deaths. In the second range-finding study, pups directly dosed with 150 mg/kg/day demonstrated biologically significant inhibition of RBC and brain cholinesterase activities. Therefore, Cheminova believes that 150 mg/kg/day is the highest dose level that could be repeatedly tested in this study, producing significant cholinergic toxicity in pups without causing death or excessive toxicity." This statement is not unreasonable, and indicates that 150 mg/kg/day would likely serve as an MTD for the add-on direct dosing of offspring component of the DNT study. Further along, Ms Allemang says: "Although the dose levels selected for the dams in this study could not be considered to reach the **Maximum Tolerated Dose (MTD)** (her words) for cholinesterase inhibition, they will provide sufficient data indicating whether pups are more sensitive to malathion-related cholinesterase inhibition than the dams." The problems with this statement include the facts that a dose level below the MTD has clearly been selected, and therefore would not be expected to plum the full magnitude of offspring versus adult susceptibility, should any be found. There is the need not only to identify offspring susceptibility should it exist, and by this point there is every evidence that a disparity of remarkable degree exists, but to determine the full magnitude of that susceptibility.

Further along in her correspondence, Ms Allemang makes a similar claim regarding dose selection in the definitive DNT study. She says "Most important was the excessive toxicity including mortality observed in pups dosed at 200 and 450 mg/kg/day in the first range-finding

study which limits the highest dose level for the definitive DNT study to less than 200 mg/kg/day." Again, this statement is not unreasonable *with regard to the add-on direct dosing of offspring component* during PND 11-21, but is *decidedly not applicable* to the definitive Guideline DNT study as is required to be conducted under Guideline OPPTS 870.6300, wherein only dams are administered the test material.

Ms Allemang goes on to say: "Cheminova would like to point out that the purpose of the definitive DNT study is to determine possible effects of malathion on the development of the central nervous system and that the target organism is the pup/very young animal, not the dam." The deficiency in this statement resides with the fact that the study should adequately assess development of the central nervous system not only in the pup/very young animal (as dosed directly) but as possibly manifested in utero and during early lactation as assessed via adequate dosing in dams. Ms Allemang goes on to say: "Therefore, testing an **MTD** (emphasis added) in the mothers would not be appropriate in this study since it would impair the main purpose of the study by eliminating the actual test subjects due to excessive toxicity and mortality." It is to be questioned whether the main purpose of this study is the add-on direct dosing of offspring component any more than the fundamental DNT study to be performed under Guideline testing requirements. It would appear both aspects are critical, and if one dose range is mutually exclusive for both components, then a higher dose range should have been employed for the definitive Guideline DNT testing component wherein only dams are administered the test material. This option was evidently provided for in the March 29, 2000 protocol. Along these lines, in my June 13 Email to Ms White, I quoted from that March 29 protocol as follows: "Gavage dose *pups* (emphasis added) from PND 11 through (presumably PND 21, text missing) at same dose/bw as prior dose to dams. Based on range-finding data, pup dose may be adjusted downward if required due to excess toxicity to pups (emphasis added)." That condition did indeed obtain in these range-finding studies, and yet the provision for exiting this obvious dosing dilemma was ignored by Ms Allemang in her April 16 response to the issues raised on March 13, 2001. This decision for dosing at but 150 mg/kg/day in the definitive studies based upon the range-finding data was unfortunate in not only precluding adequate dosing (MTD) in the Guideline DNT study component, but in leaving unaddressed the magnitude of offspring versus adult susceptibility evident in terms of acute cholinergic toxicity in the original range-finding study, which should have been recognized as of peculiar concern under FQPA. This unfortunate choice also left unaddressed the unexpected and inexplicably high toxicity among dams observed in the original range-finding data set.

There is nothing particularly more compelling in importance concerning the add-on direct dosing of offspring component that should override adequate testing in the Guideline DNT study wherein only dams are administered the test material. Hence, the second range-finding study should have included doses above 150 mg/kg/day. Since it did not, the final range-finding study was inadequate. Yet the composite of range-finding data available is adequate to predict that 150 mg/kg/day would likely be well below the MTD for dams, and would be expected to be inadequate as a high dose level for a Guideline DNT study in which only dams are dosed.

The bottom line is that the range-finding study is not acceptable, as it does not support or lead one to conclude what dose level should be employed in the conduct of the definitive DNT Guideline study. In making the decision to conduct the Guideline study at doses no higher than

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150 mg/kg/day, the registrant acknowledges in his own discussion that it would not be expected to be an *MTD* in dams. While there was a way out of this obvious dosing conflict (direct dosing of dams versus direct dosing of offspring), the registrant elected not to take the option provided in the protocol for testing offspring in the add-on component to the DNT study, i.e., at lower doses than administered to dams in the Guideline testing protocol aspect.

Since the definitive studies went forward at doses anticipated to be inadequate, the question is whether the definitive studies do or do not satisfy the Guideline testing requirements, or better yet, whether they satisfied *both* the requirements for a Guideline DNT study, wherein only dams are dosed, and the add-on direct dosing of pups component. It is both to be unanticipated and doubtful that the one dose range could satisfy both requirements based upon the range-finding studies.

DATA EVALUATION RECORD

MALATHION/057701

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT;
OPPTS 870.6300

MRID 45646401

Prepared for

Health Effects Division
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U.S. Environmental Protection Agency
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Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
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Task No. 02-40

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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|MALATHION / 057701|

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DATA EVALUATION RECORD
TXR#: 0050550

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 057701

DP BARCODE: D282204
SUBMISSION NO.: S613654

TEST MATERIAL (PURITY): Malathion (96%w/w)

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

CITATION: Fulcher, S. M. (2002) Malathion. Developmental neurotoxicity study in the CD rat by oral gavage administration. Huntington Life Sciences, Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. Laboratory report number CHV/066; 013331, March 21, 2002. MRID 45646401. Unpublished

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

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45646401), malathion (96% a.i., batch # 9010501) was administered to 24 parental female Crl:CD®BR rats per dose by gavage at dose levels of 0, 5.0, 50, or 150 mg/kg bw/day in corn oil from gestation day 6 through postnatal day 10, and to the offspring from postnatal day 11 to postnatal day 21 inclusive. A Functional Observational Battery was performed on 10 dams/dose on gestation days 12 and 18 and lactation days 4 and 10. Offspring were evaluated as follows: age-appropriate functional observation battery on days 4, 11, 21, 35, 45, and 60, automated motor activity on days 13, 17, 22, and 60; assessment of auditory startle response on days 23/24 and 60/61, assessment of learning and memory (Morris Water Maze) at postnatal days 23/24, and at postnatal day 61/62 (separate groups), brain weights on days 11, 21, and 65, and brain histopathology and morphometrics on days 21 and 65. Pup physical development was assessed by body weight. Sexual maturation of females was assessed by age of vaginal opening, and sexual maturation of males was assessed by age at completion of balano-preputial separation.

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There were no treatment-related maternal deaths before scheduled termination. Clinical signs were limited to transient post-dosing salivation (5/24 control, 4/24 at 5 mg/kg/day, 3/24 at 50 mg/kg/day, and 20/24 at 150 mg/kg/day). There were no other treatment-related effects on cholinergic signs, and there were no effects on maternal body weight, food consumption, or reproductive indices. **The maternal LOAEL for malathion in rats is 150 mg/kg/day based on an increased incidence of post-dosing salivation. The maternal NOAEL is 50 mg/kg/day.**

The **offspring NOAEL is <5 mg/kg/day** (the lowest dose tested). The **offsp. ing LOAEL is 5 mg/kg/day, based upon increased auditory startle reflex peak amplitude in PND 23/24 male and female offspring and decreased habituation in PND 60/61 females.** At 50 mg/kg/day, there was an increased incidence of slightly flattened gait in PND 60 males, and motor activity counts (rearing and ambulatory) were decreased in female pups at PND 17 and 22. At 150 mg/kg/day, additional treatment-related findings included post-dosing clinical observations on PND 17 and 18 (whole body tremors, hypoactivity, prostrate posture, partially closed eyelids, and/or abnormal gait), delayed surface righting reflex in PND 11 female pups, increased ' incidences of slightly flattened gait in PND 60 males, and increased thickness of the corpus callosum in PND 63-67 males and females.

In a companion cholinesterase inhibition study (MRID 45566201), 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 on RBC cholinesterase were noted at 5 mg/kg in males and 50 mg/kg in females following single dose acute exposures on PND 11, and at 5 mg/kg/day in both sexes on PND 21 after 11 repeated exposures. Following a single dose to young adults, effects on RBC cholinesterase 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, brain cholinesterase was inhibited 44-48% and 81-85% at 150 and 450 mg/kg, respectively, following a single (acute) dose administered on PND 11. Single doses at these levels yielded no brain cholinesterase inhibition in young adult rats. After 11 days of dosing during PND 11-21, brain cholinesterase was inhibited 16% in pups (both sexes) at 150 mg/kg/day. No inhibition of the brain enzyme was observed in young adults following 11 days of dosing. Based upon the results of the cholinesterase study, it is evident that all behavioral and neuropathological effects of treatment observed in the dams and offspring in the developmental neurotoxicity study occurred at doses at which cholinesterase was, or had been, inhibited. For acute and repeated exposures the overall LOAEL for cholinesterase inhibition was 5 mg/kg/day, based on RBC cholinesterase inhibition in PND 11 and 21 pups. The NOAEL was not determined.

This study is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6).
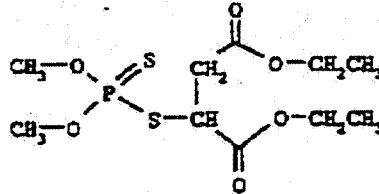
COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

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1. **Test material:** Malathion

    Description: Pale yellowish solid
    Lot/Batch #:   9010501
    Purity: 96 % a.i.
    Compound Stability: At least 1 year
    CAS # of TGAI: 121-75-5

2. **Vehicle and/or positive control:** corn oil, Mazola, Unilever Bestfoods

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

    Species: Rat
    Strain: Crl:CD®BR
    Age at study initiation: 10-11 wks
    Wt. at study initiation (mating): 204-332 g
    Source: Charles River UK Limited. Margate. Kent. England
    Housing: Individually or with litter in stainless steel grid or solid polypropylene cages
    Diet: UAR VRF1 pelleted rodent diet (Usine d'Alimentation Rationale. France).
                                    ad libitum
    Water: Tap water. *ad libitum*
    Environmental conditions: Temperature: 19-23°C
                                    Humidity: 40-70%
                                    Air changes: Up to 15/hr
                                    Photoperiod: 12 hrs dark/12 hrs light ·
    Acclimation period: At least 5 days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: March 7, 2001 (animal arrival); End: June 18, 2001 (last necropsy)

2. **Study schedule:** The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals from gestation day 6 through postnatal day 10. Pups were dosed from PND 11-21. The offspring were weaned on postnatal day 21, after which time maternal animals were killed. F1 pups remained on study until postnatal days 63-67 (study termination).

3. **Mating procedure:** Females were paired 1:1 with males of the same strain and source. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day 0. After successful mating. each pregnant female was placed into an individual cage with a solid bottom and bedding, where the dam was maintained through gestation and lactation.

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4. **Animal assignment:** Mated females were assigned to group and cage position in sequence, so that animals mated on any one day were evenly distributed among treatment groups. The allocation of mated females was adjusted so that more than one female from a given litter (as identified by the supplier) was not allocated to the same dose group. Dose groups are indicated in Table 1. Dams were assigned to neurobehavioral and neuropathological testing as shown.

Offspring were assigned to testing subgroups at the time of .itter standardization on postnatal day 4 (Table 1). One pup/sex/litter was allocated on postnatal day 4 to each of the following: motor activity, auditory startle response habituation and auditory startle pre-pulse inhibition, learning and memory at postnatal day 23/24, learning and memory at postnatal day 60, and sacrifice and brain examination on postnatal day 11.

Table 1. Study design				
	Dose (mg/kg/day)			
Experimental parameter	0	5	50	150
Maternal animals				
No. of maternal animals assigned	24	24	24	24
FOB (GD 12, 18; LD 4, 10)	10	10	10	10
Offspring				
Detailed clinical/FOB (PND 4, 11, 21, 35, 45, 60)	10/sex	10/sex	10/sex	10/sex
Motor activity (PND 13, 17, 22, 59)	10/sex	10/sex	10/sex	10/sex
Auditory startle habituation (PND 23/24, 60/61)	10/sex	10/sex	10/sex	10/sex
Learning and memory (PND 23/24, 61/62)	10/sex	10/sex	10/sex	10/sex
Brain weight PND 11 PND 21 PND 63-67	10/sex 10/sex 10/sex	10/sex 10/sex 10/sex	10/sex 10/sex 10/sex	10/sex 10/sex 10/sex
Neuropathology PND 11 PND 21 PND 63-67	10/sex 10/sex 10/sex	10/sex 10/sex 10/sex	10/sex 10/sex 10/sex	10/sex 10/sex 10/sex

5. **Dose selection rationale:** Dose levels were chosen based on the results from an oral gavage dose range-finding study (Report CHV/062; MRID 45627001) in CD rats. Results from this study are presented in a separate DER (TXR 0050550).

6. **Dosage administration:** All doses were administered once daily to maternal animals by gavage, on gestation day 6 through postnatal day 10, in a volume of 5 mL/kg of body weight. Dosing was based on the most recent body weight determination up to and including gestation day 17; the dosage volume then remained constant to postnatal day 1. Dams were not dosed during parturition. From postnatal day 1, dosing volumes were once again calculated based on the most recent body weight. Offspring were dosed by gavage in a volume of 5 mL/kg based on the most recent body weight from postnatal day 11 to postnatal day 21 inclusive, except that animals scheduled for terminal sacrifice on postnatal days 11 or 21 were not dosed on the

day of sacrifice. Controls received corn oil (vehicle) only, at the same dosing volume (5 mL/kg of body weight). The dosage level for each group was not known by the observer in the animal unit, and those involved in the necropsy, histology, or pathology.

7. **Dosage preparation and analysis:** Formulations were prepared weekly. A bulk volume of each formulation was made up each week and then divided into appropriate aliquots for administration to the appropriate groups. For each concentration. the appropriate amount of malathion was weighed out and mixed with a small amount of corn oil. After throughly mixing, the formulation was made up to the required volume using corn oil and homogenized using a high shear homogenizer. Prior to the start of the study, homogeneity and re-suspendibility of Malathion in corn oil were assessed analytically (HPLC) in trial formulations of 1 and 250 mg/mL. The concentrations of Malathion in corn oil were also determined in all formulations prepared for dosing during the first and last weeks of treatment.

Analytical Chemistry Results:
Homogeneity analysis: Concentrations of Malathion from the top, middle. and bottom of dosing preparations ranged from 0.963-1.08 mg/mL, 0.964-1.08 mg/mL, and 0.913-1.04 mg/mL, respectively, for the 1 mg/mL nominal dose. Concentrations of Malathion from the top, middle, and bottom of dosing preparations ranged from 246-270 mg/mL, 237-255 mg/mL, and 240-259 mg/mL, respectively, for the 250 mg/mL nominal dose.

Stability analysis: The mean concentrations of dosing solutions remained within 7.5-9.5% of nominal after periods of 2 days at room temperature or 15 days refrigerated.

Concentration analysis: The mean analytical concentrations of test formulations, prepared for dosing during the first and last weeks of treatment, ranged from 92.6 to 97.4% of nominal.

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. **Maternal animals:** Twice daily checks for mortality or moribundity and daily cage-side observations were conducted for maternal animals. Detailed clinical observations were conducted once weekly throughout the study. Additional observations of the dams were conducted on each day of treatment as follows: 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.

Ten dams per group were observed outside the home cage at least twice during the gestation dosing period (days 12 and 18) and twice during the lactation dosing period (days 4 and 10) prior to dosing. The following functional observations were recorded.

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Observers were unaware of the treatment group of the subjects. Subjects were also scored for ease of removal from the cage and reactivity to handling. Observations of gait, grooming, palpebral closure, posture, activity counts, rearing counts, tremors, twitches, convulsions, urination, and defecation were made for one minute in an open field (653 x 500 mm) divided into six sectors.

Individual maternal body weight data were recorded on gestation days 0, 3, 6, 10, 14, 17, and 20. During lactation, dams were weighed on postnatal days 1, 4, 7, 11, 14, 17, and 21.

Food consumption was recorded on gestation days 0-2, 3-5, 6-9, 10-13, 14-16, and 17-19 and on postnatal days 1-3, 4-6, 7-10, 11-13, 14-16, and 17-20.

From gestation day 20, dams were checked 3 times/day for evidence of parturition. They were permitted to deliver and rear offspring until postnatal day 21. Approximate numbers of live and dead offspring were recorded during the parturition process.

b. **Offspring:**

1) **Litter observations:** The day of completion of parturition was designated as lactation day (postnatal day) 0. Post-coital pup age (i.e., the age of the pup based upon the day of conception) was not reported or utilized. Pups were uniquely identified within each litter on PND 1 by toe marking. Live pups were counted, sexed and weighed individually for each litter on postnatal days 1, 4, 7, 11- 21, and 28. Daily throughout lactation, offspring were examined cage-side for numbers of live and dead pups, general clinical signs, and dam/litter interactions. Litters were examined 4 times daily for evidence of deficient maternal care. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. Additional observations were made on days of direct dosing to pups (PNDs 11-21): pre-dosing, as the animal was returned to the home cage, at the end of dosing for each group, between 1 and 2 hours after the completion of dosing of all groups, and as late as possible in the working day.

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On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were killed and discarded. On PND 11, litters were culled to 7 pups/litter, and on PND 21, litters were culled to 6 pups/litter, in order to provide either a male or female pup for necropsy at each time point.

2) **Developmental landmarks:** Beginning on postnatal day 38, male offspring were examined daily for balanopreputial separation. Beginning on postnatal day 28, female offspring were examined daily for vaginal patency. The age of onset and body weight on the day of onset were recorded.

3) **Postweaning observations:** After weaning on postnatal day 21, offspring were examined twice daily for mortality or morbidity. A full physical exam was performed weekly, up to study termination. Individual offspring body weight data were recorded weekly from postnatal day 28 until termination at postnatal day 63-67.

4) **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report.

   i. **Functional observational battery (FOB):** On postnatal days 4, 11, 21, 35, 45, and 60, a total of 10 (or at least 9) offspring/sex/group (one male or one female from each litter) was examined outside the home cage in an FOB assessment, as appropriate for the developmental stage being observed.

   Postnatal day 4: A clear arena with a floor size of 30 x 20 cm and side walls of 4.5 cm was utilized. An FOB activity sheet (paper sheet marked with concentric circles) was placed underneath the arena. The animal was then placed in the center of the FOB activity sheet and observed over a one-minute recording period. The following parameters were assessed: righting reflex, number of sections entered, maximum distance traveled, maximum pivoting angle, physical condition (e.g., skin color, physical abnormalities, cold-to-touch), locomotor coordination, and abnormal behaviors (e.g., tremors, convulsions, and excessive backward movement). The arena was disinfected between each use to prevent activity from being influenced by olfactory cues from previous rats.

   Postnatal day 11: The same arena was used as was used for postnatal day 4; however, the paper placed below the arena was divided into 9 equal segments. The animal was then placed in the center of the FOB activity sheet and observed over a one-minute recording period. The following parameters were assessed: righting reflex, number of sections entered, number of rearings, frequency of grooming, urination, physical condition (skin color, physical abnormalities, cold-to-touch), locomotor coordination, and abnormal behaviors (e.g., tremors, convulsions, and excessive backward movement). The arena was disinfected between each use to prevent activity from being influenced by olfactory cues from previous rats.

   Postnatal days 21, 35, 45, and 60: The following observations were graded and recorded. (The observations on postnatal day 21 were made after completion of

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counting, sexing, and weighing, and before the dam was removed for necropsy or before direct dosing of the offspring).

In-the-Hand Observations	Standard Arena Observations
Removal from cage	Palpebral closure
Salivation	Posture
Lacrimation	Gait
Piloerection	Tremor
Exophthalmus	Twitch
Fur condition	Convulsion
Reactivity to handling	Activity
Pupil Closure reflex (Day 35 only)	Rearing
	Grooming
	Urination
	Feces

ii. **Motor activity testing:** Motor activity was evaluated in 10 rats/sex/dose (one male or one female pup from each litter) on days 13, 17, 22, and 59. Animals were placed in plastic cages and motor activity was continuously monitored over a 1-hour period. (On day 13, it was recorded if eyes were open or closed, and on days 13 and 17, motor activity was monitored before dosing). An automated activity monitoring system collected data over successive 6-minute intervals by recording infra-red light source break frequency within the cage. Low-level beam detectors were set 3.5 cm above the cage floor to monitor ambulatory activity, while high-level beam detectors monitored rearing activity. Due to small animal size on postnatal days 13 and 17, a raised insert was placed in the cage so that activity would be detcted.

iii. **Auditory startle reflex habituation and pre-impulse inhibition of startle:** Auditory startle reflex habituation and pre-impulse inhibition of startle testing was performed on 10 offspring/sex/ dose on postnatal days 22/23 and 60/61 using an automated system. One male or one female from each litter was assigned to auditory startle testing and another male or female from the litter was assigned to pre-pulse inhibition testing.

For auditory startle habituation testing, animals were acclimated for 5 minutes to background noise. Mean startle amplitudes were recorded for 5 consecutive blocks of 10 trials for the auditory startle habituation testing. The startle stimulus consisted of 40-millisecond bursts of white noise at 90% intensity (105 dB) against a background noise level of 70 dB, with inter-stimulus intervals of 12 seconds.

For testing the pre-impulse inhibition of startle, mean startle amplitudes were recorded for 10 trials with a pre-pulse of sound immediately preceding the startle stimulus and for 10 trials without a pre-pulse. The startle stimulus consisted of 50-millisecond bursts of white noise at 100% intensity (118 dB) and the pre-pulse consisted of a 50-millisecond pulse of white noise at 70% intensity (85 dB) preceding the startle stimulus by 50 milliseconds. A total of 20 trials (10 each) were presented in a pseudo-random order with inter-trial intervals of 10, 12, 14, or 16 seconds.

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iv. **Learning and memory testing**: Learning and memory testing was performed in 10 offspring/sex/dose (one male or one female from each liter) on postnatal days 23/24 and 61/62 using a Morris water maze (a separate selected set of offspring was evaluated at each time point). A series of 3 trials was conducted on each of 4 consecutive days. The water maze consisted of a circular white plastic pool (90 cm diameter; 30 cm deep at days 23/24 and 140 cm diameter, 45 cm deep at days 61/62). The maze was filled with water at 29±3°C and made opaque with a nontoxic opacifier (Opacifer 621). A 6 cm square platform was concealed at a fixed position 1.5 cm below the surface of the water. Three starting points were identified at the edge of the pool. Each animal received 3 consecutive trials on each day of testing. For the first trial, the animal was placed on the escape platform for 30 seconds prior to testing. The animal was then placed into the water at the edge of the pool and given a maximum of 90 seconds to swim to the platform. A different starting point was used for each trial. The time to reach the platform and the number of quadrants crossed were recorded. The rat was allowed to remain on the platform for 30 seconds after each trial. If the rat did not find the platform within 90 seconds, it was placed on the platform for 30 seconds and a latency of 90 seconds was recorded.

2. Postmortem observations:

a. **Maternal animals**: Maternal animals were sacrificed by carbon dioxide inhalation on postnatal day 21. The single non-pregnant female was killed 25 days after the day of mating after having failed to produce a litter. Females who failed to rear their offspring to weaning were killed on the day the last offspring died/was killed. Adult females were subjected to a detailed macroscopic necropsy, and the number of implantation sites was recorded. The weight of the brain, pituitary, and reproductive organs (uterus and cervix, vagina, ovaries, and oviducts) was recorded. Specimens of abnormal tissues were retained in fixative, and mammary tissue was retained from females whose litters died early in lactation.

b. **Offspring**:

Animals not selected for neuropathologic evaluation: Pups culled on PND 4 or killed before PND 21 were sacrificed by i.p. injection of sodium pentobarbitone or by carbon dioxide inhalation. Offspring killed on day 11 were sacrificed by intraperitoneal injection of barbiturate. Offspring killed on day 21 or at study termination, but not selected for neuropathological evaluation, were sacrificed by carbon dioxide inhalation.

Sporadic neonatal deaths were subjected to detailed macroscopic necropsy; offspring culled on PND 4 were not necropsied. Weanling offspring and other offspring dying during or following the late lactation period were subject to detailed macroscopic necropsy, and specimens of abnormal tissue were retained in fixative. In addition, for offspring killed on day 21 or at study termination but not selected for neuropathological examination, brains were removed, weighed, and fixed in 10% neutral buffered formalin (but not examined histopathologically).

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At study termination, PND 63-67 offspring that were not selected for neuropathological evaluation were killed by carbon dioxide inhalation. A detailed macroscopic necropsy was performed. The brain was removed. weighed, and fixed in 10% neutral bufferd formalin. Additionally, where possible, the pituitary and reproductive organs from one male and one female from each litter were weighed (uterus and cervix, vagina, ovaries. testes, seminal vesicles, epididymides, and prostate, as appropriate).

Animals selected for neuropathologic evaluation: The offspring selected for brain weight or neuropathological evaluation were sacrificed on postnatal day 11, 21 or 63-67. These animals were subjected to postmortem examinations as described below.

At postnatal day 11, ten pups/sex/group (one male or one female from each litter) were selected for brain weight measurements. The head was removed and fixed for 24 hours by immersion in 10% neutral buffered formalin after opening the calvarium. Brain weights were recorded after fixation and removal of the brain from the skull. The brains from all pups of all groups were embedded in paraffin, sectioned at 4-5 μm, and stained with hematoxylin and eosin. Sections included coronal sections (olfactory lobes, forebrain, cerebrum, hippocampus. thalamus. hypothalamus, cerebrum, tectum, tegmentum, medulla oblongata) and mid-sagittal sections (cerebellum, pons). The sections were not examined microscopically.

At postnatal day 21. up to ten pups/sex/group (one male or one female from each litter) were sacrificed by intraperitoneal injection of barbiturate and perfused with gluteraldehyde and paraformaldehyde, followed by immersion in glutaraldehyde and paraformaldehyde. The brain was transected from the spinal cord above the first cervical spinal nerve. The brain length was measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum. The width was measured at the widest part of the cerebral hemispheres, and the brain was weighed.

Tissues listed below from all dose groups were embedded in paraffin and were sectioned for control and high-dose animals. Tissues were sectioned at 4-5 μm and stained with hematoxylin and eosin. Abnormalities were only sectioned for control and high-dose animals. Histopathological evaluations included the tissues listed below; only control and high-dose animals were examined microscopically.

Brain: **Coronal sections** (olfactory lobes. forebrain, cerebrum, hippocampus, thalamus, hypothalamus. tectum. tegmentum. medulla oblongata) and **mid-sagittal sections** (cerebellum, pons) were evaluated qualitatively.

The following brain morphometric measurements were performed:

Thickness of the neocortex (distance from the pial surface to the top of the white matter was measured along a line perpendicular to a tangent of the pial surface at the point where the cortex exhibits the greatest thickness)

Corpus callosum (thickness at the midline)

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Hippocampus (greatest dorsal-ventral thickness)

Cerebellum (width of the pyramis folia perpendicular to its long axis at the midpoint between the tip and base)

External germinal layer.

On postnatal day 63-67, up to 10 animals/sex/group (one male oi one female from each litter) were euthanized by i.p. injection of a barbiturate, and perfused with glutaraldehyde and paraformaldehyde (followed by immersion in glutaraldehyde and paraformaldehyde) for brain weight measurements and/or neuropathology. The brain was transected from the spinal cord above the first cervical spinal nerve. The brain length was measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum. The width was measured at the widest part of the cerebral hemispheres, and the brain was weighed. Animals were also subjected to macroscopic necropsy and abnormal tissues were preserved. The following central and peripheral nervous tissues were dissected and preserved in plastic (sciatic and tibial nerves only) or paraffin (all other tissues).

The CHECKED (X) tissues were evaluated for adult offspring.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
x	**BRAIN**	x	Sciatic nerve
			Sural nerve
	SPINAL CORD	x	Tibial nerve (knee and calf muscle branch)**
x	Cervical swelling**		Peroneal nerve
x	Lumbar swelling**	x	Lumbar dorsal root ganglia*
		x	Lumbar dorsal root fibers*
	OTHER	x	Lumbar ventral root fibers*
	Gasserian ganglion	x	Cervical dorsal root ganglia*
	Trigeminal nerves	x	Cervical dorsal root fibers*
x	Optic nerve*	x	Cervical ventral root fibers*
x	Eyes (retina) *	x	Gastrocnemius muscle (transverse section)

* longitudinal sections
** longitudinal and transverse sections

Tissues from all dose groups were embedded; however, only brains were sectioned for mid- and low-dose animals. Paraffin-embedded tissues were sectioned at 4-5 μm and stained with hematoxylin and eosin; plastic-embedded tissues were sectioned at 2 μm and stained with toluidine blue. Tissues (listed above) from control and high-dose animals were examined microscopically.

Detailed morphometric evaluation of the neocortex, hippocampus, and cerebellum was conducted as follows:

Thickness of the neocortex (distance from the pial surface to the top of the white matter was measured along a line perpendicular to a tangent of the pial surface at the point where the cortex exhibits the greatest thickness)

Corpus callosum (thickness at the midline)

Hippocampus (greatest dorsal-ventral thickness)

Cerebellum (width of the pyramis folia perpendicular to its long axis at the midpoint between the tip and base)

External germinal layer (measured at day 21: During processing of tissues from PND 21 offspring, one layer of cassettes was not removed from the processing machine in error. These tissues subsequently went through the clean cycle of the machine, after which the error was detected and the affected tissues were reprocessed. Control and high-dose tissues were examined by the study pathologist and no discernable difference was noted between control and treated tissues. Therefore, the study authors did not consider the integrity of the study to be affected by the error).

D. DATA ANALYSIS:

1. **Statistical analyses:** Statistical analyses were performed on the following parameters: gestation body weight and body weight change, lactation body weight and body weight change, gestation food consumption, lactation food consumption, litter size, offspring survival indices, offspring body weight and body weight change, body weight change for offspring selected for behavioral testing, activity and rearing counts for maternal FOBs, and offspring FOB parameters, motor activity data, startle response data, and water maze data.

The following statistical methodology was used: If 75% of the data (across all groups) were the same value, then a frequency analysis was applied. Treatment groups were compared using a pairwise Fisher's Exact test to compare each dose group to the control. If Bartlett's test for homogeneity was not significant at the 1% level, then parametric analysis was utilized. If the F1 test for monotonicity of dose-response was not significant at the 1% level, William's test for a monotonic trend was applied. If the F1 test was significant, Dunnett's test was applied.

If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then nonparametric tests were applied. If the H1 Test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was applied. If the H1 Test was significant, Steel's test was performed.

If ANOVA (or the nonparametric equivalent) was not significant, then significant results of inter-group comparison with the control were not reported.

2. **Indices:**

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a. __Reproductive indices__: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Gestation index = (Number 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-implantation survival index = (Total No. of offspring born/Total No. of implantation sites) × 100

Live birth index = (Number of live offspring at PND 1/Total number of offspring born) × 100

Viability index = (Number of live offspring at PND 4 precull/Number of live offspring at PND 1) ×100

Lactation index = (Number of live offspring on day of examination/Number of live offspring on PND 4 after culling) × 100

3. __Positive and historical control data__: Positive control data from the performing laboratory were previously submitted to EPA. A review of these data are appended to this DER. No historical control data were submitted in relation to this study.

II. RESULTS

A. PARENTAL ANIMALS

1. __Mortality and clinical and functional observations__: There were no maternal deaths before scheduled termination. No reported maternal clinical observations were suggestive of treatment-related lack of maternal care of litters. General clinical signs in dams consisted primarily of brown or yellow staining, hair loss, and encrustation, mostly located on the head, forelimbs, and thorax. These findings occurred in almost all dams in the control and treated groups (23/24 control, 23/24 at 5 mg/kg/day, 24/24 at 50 mg/kg/day, and 23/24 at 150 mg/kg/day) (MRID 45646401, Appendix 1, pp. 167-174). The study author considered these findings to be related to the use of corn oil as the vehicle.

The only other notable finding was post-dosing salivation which was observed in all groups: 5/24 in control, 4/24 at 5 mg/kg/day, 3/24 at 50 mg/kg/day, and 19/24 at 150 mg/kg/day. For the control through 50 mg/kg/day groups, the post-dose salivation was characterized as occurring one time (except for one control dam with 2 occurrences and one 5 mg/kg/day dam with 4 occurrences), almost entirely during the gestation period. In the 150 mg/kg/day group, post-dosing salivation was not observed until after several days of dosing had been completed, was generally noted on multiple days of dosing (only four of the dams had a single incident reported), and often was observed during both the gestation and lactation periods (MRID 45646401, Appendix 2, pp. 175-178). The study author attributed the post-dosing salivation to distaste of the formulation rather than an adverse effect of treatment; however, no

arguments were provided to substantiate this claim. In fact. the data support the conclusion that the observed salivation was most likely not related to taste aversion. since salivation did not occur on the first day of dosing (which would have been expected with an aversion to taste). but commenced after several days of dosing had been completed. This is consistent with the observation that the LOAEL for erythrocyte cholinesterase inhibition is 50 mg/kg/day in adult rats following 11 repeated doses of malathion, while it is 450 mg/kg with a single dose of malathion (comparative cholinesterase study: MRID 45566201). It is also noted that post-dosing salivation was observed in other studies with malathion. In the dose range-finding developmental neurotoxicity study with malathion (MRID 45627001), post-dosing salivation was observed at 75 and 150 mg/kg/day. as well as at higher dose levels. Additionally, in the comparative cholinesterase study with malathion (MRID 45566201), post-dosing salivation was observed in dams dosed from GD6-LD10 at 150 mg/kg/day at a similar incidence to that seen in the developmental neurotoxicity study. The data from the comparative cholinesterase study demonstrated that repeated doses of malathion at 50 and 150 mg/kg/day resulted in significant treatment-related decreases in red blood cell cholinesterase in young adult rats and GD 20 dams. Therefore, it is biologically and toxicologically plausible that the observed post-dosing salivation was an adverse effect of treatment.

No treatment-related functional observations were noted in maternal animals evaluated on gestation days 12 and 18 and lactation days 4 and 10. The study report stated that on gestation day 12. higher grades of activity were observed in treated dams during removal from the home cage; however. the data (MRID 45646401, Table 2, pp. 81) did not support this assertion. There were no apparent treatment-related effects on observations recorded during handling or during observations on animals in an open field arena. including possible effects on cholinergic signs (e.g.. urination or tremors). Piloerection was noted in some treated animals on GD 12. GD 18, PND 4, and PND 10 and in controls on PND 4. Since no dose-response relationship was observed. the effect is not considered test substance-related.

2. **Body weight and food consumption**: Selected group mean body weights and food consumption values for pregnant or nursing dams are summarized in Table 2. There were no significant treatment-related effects on body weight. body weight gain. or food consumption during gestation or lactation.

TABLE 2. Selected Mean (±SD) Maternal Body Weight and Food Consumption[a]				
	Dose (mg/kg/day)			
Observations/study interval	0	5	50	150
Gestation (n= 24)				
Body wt. Gestation day 0 (g)	258±19	261±23	256±27	260±29
Body wt. Gestation day 6 (g)	290±21	293±26	287±29	294±29
Body wt. Gestation day 14 (g)	340±22	341±25	336±32	347±35
Body wt. Gestation day 20 (g)	426±28	430±33	420±40	437±45
Wt. gain gestation days 0-6 (g)	32±6	33±7	30±5	34±6

TABLE 2. Selected Mean (±SD) Maternal Body Weight and Food Consumption[a]				
Observations/study interval	Dose (mg/kg/day)			
	0	5	50	150
Wt. gain gestation days 6-20 (g)	135±14	137±13	133±16	143±20
Food consumption gestation days 0-2 (g/animal/day)	27±3	28±3	27±3	28±2
Food consumption gestation days 6-9 (g/animal/day)	26±3	26±3	26±3	27±3
Food consumption gestation days 17-19 (g/animal/day)	27±3	27±3	27±3	28±3
Lactation (n=21-24)				
Body wt. lactation day 1(g)	330±25	327±23	327±29	337±38
Body wt. lactation day 11 (g)	363±24	367±23	367±30	373±31
Body wt. lactation day 21 (g)	361±21	366±26	352±34	367±31
Wt. gain lactation days 1-11 (g)	33±11	41±14	40±10	37±14
Wt. gain lactation days 1-21 (g)	31±13	39±17	25±16	30±14
Food consumption lactation days 1-10 (g/animal/day)	44±4	47±7	45±5	45±4
Food consumption lactation days 11-20 (g/animal/day)	71±8	74±6	70±8	71±7

a Data obtained from Tables 10-15 pages 89-94. MRID 45646401.

3. **Reproductive performance:** Results for the maternal animals are summarized in Table 3. There were no treatment- related effects on length of gestation, gestation index, the parturition process, or implantation rate. As noted above, there was no indication of treatment-related maternal toxicity or clinical signs, which might contribute to a lack of maternal care of pups during lactation; signs indicative of poor maternal care were not noted in the study report.

TABLE 3. Reproductive Performance [a]				
Observation	Dose (mg/kg/day)			
	0	5	50	150
Number mated	24	24	24	24
Number Pregnant	24	24	23	24
Total litter loss (post partum)	0	1	2	1
Mean gestation duration (days)	22.4	22.3	22.4	22.4
Mean (±SD) implantations/dam	16.6±1.8	16.1±1.9	15.0±1.9	16.2±1.8
Gestation index (%)	100	100	100	100

a Data obtained from Tables 1. 16. &21. pages 80. 95. & 100. MRID 45646401.

4. **Maternal postmortem results:** No treatment-related effects were noted upon macroscopic examination at necropsy. There were no treatment-related effects on absolute or relative brain weight or on pituitary, uterus and cervix, or vaginal weights. Dams with total litter mortality (one at 5 mg/kg/day - PND 9, two at 50 mg/kg/day - PND 4 and 14, and one at 150 mg/kg/day - PND 13) were sacrificed on the day of litter loss. The mammary tissue of the 5 mg/kg/day dam and of the 50 mg/kg/day dam that was killed on lactation day 4 were observed to be pale and inactive at necropsy.

B. OFFSPRING:

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1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 4. There was no difference in litter size among groups at birth, no treatment-related effects on the post-implantation survival index or sex ratio and no effect of treatment on pup survival for litters reared to weaning. The apparent decrease in survival on PND 21 is due to the removal of one offspring/litter for an interim kill (for neuropathological examination) on PND 11.

   Four treated litters (1 at 5 m/kg/day, 2 at 50 mg/kg/day, and 1 at 150 mg/kg/day) did not survive to weaning. As described above, total litter loss was reported on PND 9 in the 5 mg/kg/day litter, PND 4 and 14 in the two 50 mg/kg/day litters, and PND 13 in the 150 mg/kg/day litter. In the absence of a clear dose-response relationship and in the absence of any effects on pup survival for litters reared to weaning, this finding is of questionable toxicological significance.

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TABLE 4. Litter size and viability [a]				
Observation	Dose (mg/kg/day)			
	0	5	50	150
Number of litters born	24	24	23	24
Total number born	369	375	344	374
Deaths on PND 1	1	8	3	2
Sex Ratio Day 1 (% ♂)	47.3	54.1	52.8	50.0
Total litter size	15.4±2.0	15.4±2.0	15.0±2.1	15.4±1.8
Mean litter size:				
Day 1	15.0±1.9	15.3±2.0	15.0±2.2	15.2±1.9
Day 4 [b]	14.6±2.1	15.0±2.0	14.5±1.6	15.0±2.0
Day 4 [c]	8.0±0.0	8.0±0.0	8.0±0.0	8.0±0.0
Day 11	7.9±0.3	7.9±0.3	7.8±0.7	7.9±0.3
Day 17 [d]	6.9±0.3	6.8±0.7	6.8±0.7	6.8±0.7
Day 21 [d]	6.8±0.4	6.8±0.7	6.8±0.7	6.8±0.7
Post-implantation survival index (%)	94.0	95.8	94.2	95.2
Live birth index (%)	97.9	99.2	99.4	98.9
Viability index	97.2	97.8	97.7	98.8
Lactation index:				
Day 7	99.5	98.9	98.2	98.9
Day 11	98.4	98.9	97.0	98.9
Day 21 [d]	85.4	85.3	84.5	84.8

a Data obtained from Tables 21-23. pages 100-102. and Appendix 19. pages 332-335. MRID 45646401.
b Before standardization (culling).
c After standardization (culling).
d Interim sacrifice of one offspring/litter on PND 11.
Note: These data are representative of pups that continued into neurobehavioral and neuropathological assessement. Because there were few litters with 100% mortality. these summary values do not misrepresent the overall toxicological response of the offspring.

There were no apparent treatment-related clinical signs noted in offspring prior to direct treatment starting on PND 11. Following the initiation of direct dosing procedures, the following observations were noted. Four offspring (i.e., all surviving pups from a single litter) in the 150 mg/kg/day group exhibited whole body tremors and hypoactivity after dosing on PND 17 and 18. Two of these pups also exhibited prostrate posture and partially closed eyelids on PND 17 and another of these pups showed abnormal gait on PND 19. At 50 mg/kg/day, observations were limited to transient post-dosing salivation in one pup on PND 21. At 5 mg/kg/day. observations were limited to transient post-dosing salivation in one pup on PND 19. No other treatment-related clinical signs were reported.

2. **Body weight:** No treatment-related effects on body weight or body weight gain were observed prior to weaning. including during the period that pups were being directly dose with malathion (i.e., PND 11-21). Selected mean preweaning pup body weight data are presented in Table 5.

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TABLE 5. Mean (±SD) Pre-weaning Pup Body Weights and Body Weight Gain (g) [a]								
Postnatal Day	Dose (mg/kg/day)							
	0	5	50	150	0	5	50	150
	Males				Females			
N	24	23	21	23	24	23	21	23
1	6.5±0.6	6.6±0.7	6.7±0.8	6.5±0.5	6.1±0.6	6.2±0.6	6.2±0.6	6.1±0.5
4 b	8.7±1.2	9.1±1.4	9.0±1.6	8.6±1.1	8.2±1.3	8.6±1.3	8.6±1.4	8.1±1.0
4 c	8.7±1.3	9.1±1.4	9.2±1.5	8.6±1.1	8.2±1.3	8.6±1.3	8.6±1.4	8.2±1.0
11	24.0±4.1	25.1±3.5	25.1±4.4	23.8±3.0	23.1±4.3	23.9±3.4	23.9±4.0	23.0±3.1
17	40.8±5.5	43.3±3.9	41.6±6.5	41.4±4.2	39.8±6.0	41.5±3.6	40.3±5.7	39.9±3.9
21	51.8±7.5	55.6±6.2	53.4±8.2	53.2±5.6	50.1±7.8	52.6±5.4	51.5±6.7	50.6±5.5
Weight gain Days 1-4	2.2±0.9	2.5±0.8	2.5±0.8	2.1±0.8	2.1±0.8	2.4±0.7	2.4±0.9	2.1±0.7
Weight gain Days 1-11	17.4±3.8	18.5±3.0	18.4±3.8	17.3±2.9	17.0±3.8	17.7±2.9	17.6±3.5	16.9±2.9
Weight gain Days 11-21	28.9±6.0	30.5±3.4	28.3±4.1	29.4±3.5	28.0±5.8	28.7±2.5	27.7±3.1	27.6±3.1
Weight gain Days 1-21	45.3±7.2	49.0±5.7	46.8±7.5	46.7±5.3	44.0±7.4	46.4±4.8	45.3±6.2	44.5±5.2

a Data obtained from Tables 24-27. pages 103-106. MRID 45646401.
b Before standardization (culling).
c After standardization (culling).

No treatment-related effects on postweaning body weights or body weight gain were observed. Selected mean postweaning offspring body weight data are presented in Table 6.

TABLE 6. Mean (±SD) Post-weaning Pup Body Weights and Body Weight Gain (g) [a]								
Postnatal Day	Dose (mg/kg/day)							
	0	5	50	150	0	5	50	150
	Males				Females			
N	70	69	62	66	70	66	60	68
35	140±18	147±18	145±19	144±15	124±15	129±13	126±15	126±12
49	270±29	280±28	274±29	277±25	194±17	199±18	197±18	196±14
56	335±34	345±33	339±33	345±30	223±20	228±20	227±21	224±17
63	386±37	395±37	392±36	398±34	246±22	247±21	247±23	245±18
21-63	334±31	340±33	338±31	345±30	196±18	195±18	195±19	195±15

a Data obtained from Tables 47-50. pages 136-139. MRID 45646401.

3. Developmental landmarks:

a. **Sexual maturation:** There were no treatment-related effects on the mean age for attainment of vaginal opening for females or preputial separation for males. The data are presented in Table 7. Body weights at sexual maturation were also similar among groups.

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TABLE 7. Mean (±SD) Age at Sexual Maturation (days) [a]			
Parameter	Dose (mg/kg/day)		
	0	5	50	150
N (M/F)	70/70	69/66	62/60	65/68
Preputial separation (males)	45.0±2.6	44.2±2.1	44.8±2.7	45.0±2.5
Vaginal opening (females)	35.3±2.8	35.1±2.3	35.3±2.3	35.2±2.4

a Data obtained from Tables 51 & 52. pages 140 &141. MRID 45646401.

4. Behavioral assessments:

a. **Functional observational battery**: Selected functional observational data from testing in an arena are summarized in Table 8. The mean surface righting score for PND 11 female pups at 150 mg/kg/day was increased (1.6) as compared to control females (1.0). This change was attributed to 4/10 150 mg/kg/day female pups which had slow (3 to 5 seconds) surface righting and 1/10 female that failed (>5 seconds), compared with 0/10 slow or failed female pups in the control group. Rearing scores for males and females treated with 50 mg/kg/day were significantly increased on PND 45 compared to controls; however, in the absence of a dose-response relationship (no effects were noted at 150 mg/kg/day), this effect was not considered toxicologically significant. On PND 60, a slightly flattened gait was noted in 3/10 mid-dose (50 mg/kg/day) males and 6/10 high-dose (150 mg/kg/day) males and was considered to be treatment-related. Prior to PND 60, sporadic incidences of flattened gait were observed in both sexes. in all groups including control, and at all time points (i.e., PND 21, 35, and 45). At PND 21, a slightly flattened gait was observed in 3/10 females at 150 mg/kg/day but was not judged to be treatment-related. due to the presence of a background incidence of this finding in controls. An increase in the incidence of PND 60 females exhibiting a slightly elevated gait was observed at 50 mg/kg/day (4/10) and at 150 mg/kg/day (3/10); however, the lack of a dose relationship suggested that this finding was not related to treatment. There were no incidences of elevated gait in males. No other treatment-related effects were noted in arena observations or functional observations of hand-held animals.

TABLE 8. Functional Observational Battery Results (incidence) [a]							
	Dose (mg/kg/day)						
	0	5	50	150	0	5	50	150
	Males				Females			
Surface righting reflex (mean-scale of 1 to 3)								
-PND 4 (mean)	2.4	2.2	2.5	2.3	2.6	2.8	2.7	2.5
-PND 11 (mean)	1.1	1.2	1.1	1.1	1.0	1.4	1.2	1.6
Grade 1 - immediate	9	8	10	9	10	7	9	5
Grade 2 - slow, 3-5 sec	1	2	1	1	0	2	2	4
Grade 3 - fail. >5 sec	0	0	0	0	0	1	0	1
Maximum pivoting angle (mean)								
-PND 4	54.0	54.0	63.0	72.0	58.5	85.5	31.5	54.0
Maximum distance traveled (mean- cm)								
-PND 4	0.6	0.4	1.0	1.4	0.8	1.4	1.2	0.6
Activity (mean # of sections entered)								
-PND 4	0.9	0.7	1.5	1.9	1.0	1.6	1.0	1.0
Activity count (mean)								
-PND 11	11.3	11.2	12.1	12.4	13.0	10.7	12.1	11.8
-PND 21	3.9	6.1	7.2	4.8	6.1	4.9	8.7	5.1
-PND 35	8.4	10.9	9.0	10.0	11.3	12.9	10.0	12.2
-PND 45	10.0	9.3	10.7	9.8	12.5	16.2	15.3	11.0
-PND 60	8.4	9.4	8.7	9.5	17.1	20.0	17.8	15.2
Rearing count (mean)								
-PND 11	0.0	0.2	0.1	0.1	0.1	0.0	0.2	0.3
-PND 21	0.9	2.7	2.6	1.9	2.3	1.9	3.3	1.9
-PND 35	2.7	5.2	3.6	3.9	4.7	4.7	4.6	4.4
-PND 45	1.9	2.3	4.5	2.1	3.5	4.9	5.9*	2.3
-PND 60	3.6	4.3	3.3	3.0	7.9	9.4	8.9	7.2
Flattened gait (grade=slight)								
-PND 21	1	1	0	1	1	0	0	3
-PND 35	0	1	0	1	0	1	0	1
-PND 45	1	1	1	1	0	0	0	0
-PND 60	0	1	3	6	0	0	0	1
Elevated gait (grade=slight)								
-PND 21	0	0	0	0	0	0	0	1
-PND 35	0	0	0	1	0	1	0	1
-PND 45	0	0	1	0	1	1	1	1
-PND 60	0	0	0	0	1	2	4	3

a   Data obtained from Tables 28-33, pages 107-112, MRID 45646401.

N = 9-11/sex/dose

* Statistically different from control, p<0.05.

b. Motor activity: Total activity data are presented in Tables 9 and 10 for rearing and ambulatory activity, respectively.

On PND 13, rearing and ambulatory activities were increased for males and females at all dose levels compared to controls. However, this effect at PND 13 was judged to be of questionable toxicological significance due to the large variations in the data (i.e., CVs generally exceeding 200%) and to the low baseline activity values. No other motor activity effects were noted for males at PND 17, 22, or 59. On PND 17 and 22, decreased rearing and cage floor activity were noted for treated females compared to controls. A dose response was observed on both days, and the effect reached statistical significance ($p < 0.05$ or 0.01) for cage floor activity at 50 and 150 mg/kg/day at PND 22. The decreased cage floor activity noted for PND 17 and 22 females at 50 and 150 mg/kg/day (Table 10) is supported by the decreased rearing activity at the same ages and doses (Table 9), and is considered an effect of treatment at those doses. No other significant motor activity effects were noted for females.

Habituation of activity within the session became much more pronounced on postnatal days 22 and 59 in comparison to days 13 and 17, consistent with typical motor system maturation in the rat.

TABLE 9. Mean (±S.D.) Motor Activity Data: Rearing (High Beam Breaks) (total activity counts for session) [a]				
Test Day	Dose (mg/kg/day)			
	0	5	50	150
Males				
PND 13	0.6±1.3	1.1±2.5 (183%)	3.1±6.0 (517%)	3.7±8.1 (617%)
PND 17	44.1±58.5	105.1±104.7	75.9±94.1	49.2±55.9
PND 22	34.6±14.1	59.5±29.9	34.5±21.0	55.1±61.1
PND 59	290.0±93.2	400.3±176.8	268.1±93.2	313.9±157.2
Females				
PND 13	0.1±0.3	0.5±1.0 (500%)	0.5±1.2 (500%)	1.6±3.9 (1600%)
PND 17	59.7±83.8	92.6±115.0	30.1±31.9 (50%)	39.9±61.3 (67%)
PND 22	60.9±36.4	33.5±15.2 (55%)	32.0±24.7 (53%)	33.5±34.0 (55%)
PND 59	296.3±69.0	296.1±93.1	290.1±99.0	318.0±126.1

a Data obtained from Tables 37-40. pages 116-123. MRID 45646401.
N = 9-10/sex/dose. Number in parentheses is % of control, calculated by reviewer.

TABLE 10. Mean (±S.D.) Motor Activity Data: Cage Floor Activity (Low Beam Breaks) (total activity counts for session) [a]			
Test Day	**Dose (mg/kg/day)**		
	0	**5**	**50**	**150**

	0	**5**	**50**	**150**
Males				
PND 13	217.0±216.1	248.3±166.0	258.3±222.0	281.8±199.8
PND 17	401.2±380.9	582.6±444.6	431.4±367.1	594.2±590.7
PND 22	202.3±71.2	276.6±136.0	233.0±125.2	278.3±219.9
PND 59	1150.9±305.2	1165.7±240.2	1014.3±334.0	1134.8±333.7
Females				
PND 13	235.2±156.8	121.5±75.5	188.8±189.8	229.2±122.5
PND 17	437.0±495.8	643.8±450.7	305.1±204.1 (70%)	210.4±194.9 (48%)
PND 22	343.3±173.4	243.1±131.9 (71%)	187.7*±120.9 (55%)	182.1**±82.3 (53%)
PND 59	1523.9±408.5	1340.8±347.9	1412.2±440.5	1590.8±520.8

a Data obtained from Tables 37-40. pages 116-123. MRID 45646401.
N = 9-10/sex/dose. Number in parentheses is % of control. calculated by reviewer.
*p<0.05. **p<0.01

c. **Auditory startle reflex habituation**: The auditory startle reflex peak amplitude data are shown in Table 11. Data on pre-pulse reflex inhibition are shown in Table 12. Mean startle amplitudes for treated offspring were higher than controls for both sexes on PND 23/24 in all five testing blocks. Mean amplitudes reached statistical significance for PND 23/24 females at 5 mg/kg/day (block 5). 50 mg/kg/day (blocks 4 and 5), and 150 mg/kg/day (block 5). Although a clear dose-response relationship was not established. since the magnitude of response was generally equivalent across all treated groups, the overall consistency of the effect throughout all testing sessions and at all treatment levels was considered particularly notable. It is possible that this similarity of response magnitude across the treated groups was due to a ceiling effect. The control values for the PND 23/24 males and females were quite , similar to the control (and treated groups, which did not demonstrate any adverse effect of treatment) values reported for another developmental neurotoxicity study with dimethoate that was conducted in the same laboratory (MRID 45529703), demonstrating that the increases in peak amplitude in the treated groups were not attributable to aberrant control data. At PND 60/61, group mean startle amplitudes for treated females were lower than controls, especially during the first two blocks. No effects were noted for male offspring at PND 60/61. The change in mean peak amplitude across testing sessions for PND 60/61 males at 150 mg/kg/day and females at all treatment levels was notably less than the control values, indicating a treatment-related effect on habituation. No consistent differences in the degree of pre-pulse inhibition were seen in either sex at any dose.

	Trial Block	Dose (mg/kg/day)			
		0	5	50	150
Males					
PND 23/24	1	164.8±40.9	231.9±79.9	210.2±62.2	236.4±122.0
	2	175.6±38.3	199.6±76.5	202.8±68.3	203.5±155.2
	3	146.0±43.6	174.1±63.6	170.9±60.5	203.8±128.7
	4	145.5±59.4	155.4±59.3	169.2±52.0	177.3±124.1
	5	136.2±48.4	171.5±54.3	157.3±46.7	179.6±116.4
	Change b	28.6	60.4	52.9	56.8
PND 60/61	1	48.1±15.3	52.8±27.9	50.2±19.5	42.6±17.5
	2	35.8±7.9	44.9±21.5	37.2±13.2	35.8±18.0
	3	36.9±11.4	39.6±21.6	33.6±7.3	33.3±18.5
	4	32.3±8.9	31.5±10.5	29.0±6.0	31.1±12.4
	5	34.7±12.8	35.5±14.3	31.8±9.7	34.4±19.6
	Change	13.4	17.3	18.4	8.2
Females					
PND 23/24	1	177.0±57.2	222.3±65.9	229.8±59.5	217.4±76.9
	2	141.2±59.2	182.1±72.6	199.9±71.5	180.8±92.6
	3	134.1±44.4	167.2±66.3	185.9±64.4	147.8±67.2
	4	124.1±49.1	156.2±40.8	220.1***±66.6	155.8±54.6
	5	110.9±26.9	158.9*±53.2	200.7**±87.7	154.6*±35.4
	Change	66.1	63.4	29.1	62.8
PND 60/61	1	45.6±29.6	31.7±10.8	31.0±7.2	29.9±11.7
	2	36.4±21.6	26.3±9.1	25.4±7.5	25.8±9.2
	3	31.5±17.3	27.0±11.1	29.1±11.1	25.1±11.7
	4	30.1±17.6	23.4±6.8	28.4±8.3	23.8±9.8
	5	29.6±18.0	24.6±8.0	26.2±11.4	26.1±11.9
	Change	16.0	7.1	4.8	3.8

TABLE 11. Auditory Startle Reflex Peak Amplitude Data (mean ±S.D.) [a]

a Data obtained from Tables 43 & 44, pages 128-131, MRID 45646401.
b Change = mean value of first session minus mean value of last session.
N = 9-10/sex/dose. *p<0.05; **p<0.01; ***p<0.001.

TABLE 12. Auditory Startle Reflex Pre-Pulse Inhibition Peak Amplitude Data (mean ±S.D.) [a]					
		Dose (mg/kg/day)			
		0	5	50	150
Males					
PND 23/24	Stimulus without pre-pulse	275.8±77.2	285.8±64.3	288.1±64.7	317.4±94.4
	Stimulus with pre-pulse	201.6±64.7	236.9±45.0	210.1±59.9	237.0±83.6
	% Inhibition	26.6±11.9	15.7±13.2	27.1±12.2	25.4±16.2
PND 60/61	Stimulus without pre-pulse	69.9±18.9	69.8±27.2	59.3±14.9	79.1±31.2
	Stimulus with pre-pulse	38.4±13.0	49.2±24.2	37.2±11.3	52.7±28.1
	% Inhibition	42.5±19.1	30.0±21.3	36.6±10.5	33.8±16.4
Females					
PND 23/24	Stimulus without pre-pulse	209.4±54.7	325.1±96.1	264.8±108.4	269.9±119.5
	Stimulus with pre-pulse	172.7±47.3	247.6±70.8	216.2±107.2	230.2±113.5
	% Inhibition	17.0±13.3	23.4±10.6	18.6±16.6	23.2±12.1
PND 60/61	Stimulus without pre-pulse	53.0±8.0	76.7**±19.7	59.1±20.5	66.6±21.0
	Stimulus with pre-pulse	30.8±9.9	48.1±18.1	38.8±21.4	44.1±19.0
	% Inhibition	40.4±19.8	37.4±17.8	36.7±22.4	34.3±17.3

a Data obtained from Tables 45 & 46. pages 132-135. MRID 45646401.
N = 10/sex/dose. **p<0.01

d. Learning and memory testing: There were no treatment-related effects on performance in the Morris water maze at either time point. Data are summarized in Tables 13 and 14. For all groups, in both sexes, and at both ages tested, mean trial times and error rates decreased over the days of testing.

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TABLE 13. Morris Water Maze Performance - Males (mean ± S.D.) [a]				
	Dose (mg/kg/day)			
Test Day/Parameter	**0**	**5**	**50**	**150**
PND 23/24				
Test day 1 — Trial time (sec)	71.9±16.0	67.2±19.6	69.0±18.3	74.6±11.4
Test day 1 — No. failed trials	1.9±0.9	1.5±1.0	1.7±1.1	1.5±1.2
Test day 1 — No. sector entries	19.6±4.7	16.5±3.4	17.3±4.4	18.8±1.9
Test day 2 — Trial time (sec)	41.0±22.8	54.0±17.5	39.9±16.2	42.7±18.6
Test day 2 — No. failed trials	0.5±0.7	0.8±0.9	0.4±0.5	0.8±0.8
Test day 2 — No. sector entries	12.0±4.7	13.8±3.9	10.8±4.0	11.3±5.3
Test day 3 — Trial time (sec)	38.8±17.9	46.4±17.2	38.8±19.2	36.3±12.9
Test day 3 — No. failed trials	0.3±0.5	0.9±0.7	0.3±0.7	0.3±0.5
Test day 3 — No. sector entries	11.7±4.8	12.9±3.2	10.4±4.3	10.0±3.9
Test day 4 — Trial time (sec)	25.0±14.9	25.6±10.9	28.0±24.6	21.3±8.1
Test day 4 — No. failed trials	0.2±0.4	0.1±0.3	0.4±0.8	0.0±0.0
Test day 4 — No. sector entries	7.6±3.3	7.7±3.0	7.9±4.9	6.5±2.1
PND 61/62				
Test day 1 — Trial time (sec)	69.7±12.6	72.4±13.6	68.4±18.9	67.0±15.9
Test day 1 — No. failed trials	1.5±0.8	1.9±0.7	1.8±1.0	1.4±0.9
Test day 1 — No. sector entries	15.3±2.8	15.4±4.1	14.8±4.0	14.9±4.8
Test day 2 — Trial time (sec)	34.3±18.4	41.6±16.2	42.2±19.9	36.7±18.9
Test day 2 — No. failed trials	0.4±0.7	0.3±0.5	0.6±0.7	0.3±0.7
Test day 2 — No. sector entries	9.5±4.0	10.4±3.3	10.7±4.4	10.4±5.7
Test day 3 — Trial time (sec)	27.4±19.9	22.5±10.7	26.7±14.2	23.0±12.0
Test day 3 — No. failed trials	0.2±0.6	0.0±0.0	0.1±0.3	0.1±0.3
Test day 3 — No. sector entries	6.5±3.3	6.0±2.3	6.9±3.1	6.4±3.6
Test day 4 — Trial time (sec)	25.1±14.0	23.1±18.4	20.1±17.1	17.5±9.9
Test day 4 — No. failed trials	0.0±0.0	0.2±0.4	0.1±0.3	0.1±0.3
Test day 4 — No. sector entries	6.8±3.3	6.7±4.2	5.7±4.0	5.5±3.1

a Data obtained from Tables 41 & 42. pages 124 & 126. MRID 45646401.
N = 10/sex/dose

TABLE 14. Morris Water Maze Performance - Females (mean ± S.D.) [a]				
Test Day/Parameter	**Dose (mg/kg/day)**			
	0	**5**	**50**	**150**
PND 23/24				
Test day 1 — Trial time (sec)	72.2±15.8	69.3±11.9	60.4±24.3	69.5±15.4
No. failed trials	2.0±0.9	1.8±0.6	1.4±1.3	1.3±1.2
No. sector entries	19.0±3.4	18.0±2.4	17.3±6.3	18.5±3.4
Test day 2 — Trial time (sec)	51.6±18.2	46.9±22.6	50.5±27.3	40.9±14.5
No. failed trials	0.9±0.9	0.7±0.8	0.9±1.2	0.2±0.4
No. sector entries	15.7±4.4	12.3±5.5	14.7±7.6	12.8±4.2
Test day 3 — Trial time (sec)	39.1±21.2	28.8±12.7	34.3±23.1	26.5±8.0
No. failed trials	0.5±0.7	0.1±0.3	0.4±1.0	0.1±0.3
No. sector entries	11.5±4.5	8.9±3.2	11.6±7.8	9.1±2.7
Test day 4 — Trial time (sec)	27.6±19.3	34.1±14.0	34.7±23.1	24.6±23.0
No. failed trials	0.3±0.5	0.3±0.7	0.5±1.0	0.2±0.6
No. sector entries	8.6±4.5	10.6±3.5	11.1±8.0	8.4±5.9
PND 61/62				
Test day 1 — Trial time (sec)	69.4±17.1	70.5±16.0	69.2±18.5	72.1±11.5
No. failed trials	1.8±1.0	1.7±0.8	1.2±0.8	1.7±0.7
No. sector entries	17.0±5.1	15.9±2.6	15.0±4.5	16.4±2.3
Test day 2 — Trial time (sec)	47.7±19.0	45.7±21.9	36.5±17.9	45.8±21.2
No. failed trials	0.5±0.7	0.7±0.9	0.2±0.4	0.9±1.0
No. sector entries	12.6±4.8	13.2±5.2	9.5±4.0	11.8±4.9
Test day 3 — Trial time (sec)	30.0±25.3	37.3±22.9	26.7±14.3	24.9±9.8
No. failed trials	0.3±0.9	0.5±0.8	0.1±0.3	0.0±0.0
No. sector entries	7.7±5.0	9.4±5.3	7.6±3.3	7.2±2.1
Test day 4 — Trial time (sec)	26.8±8.8	32.9±24.8	23.6±13.8	21.4±6.9
No. failed trials	0.0±0.0	0.3±0.9	0.2±0.4	0.0±0.0
No. sector entries	8.4±2.6	9.4±5.9	7.0±2.7	7.0±3.3

a Data obtained from Tables 41 & 42. pages 125 & 127. MRID 45646401.
N = 10/sex/dose

5. Postmortem results:

1) **Unscheduled deaths**: Necropsies were conducted on some of the F1 animals that died or were sacrificed for humane reasons prior to scheduled sacrifice. Although there were scattered findings (not presented in DER). none appeared to be common among treated animals.

2) **Animals selected for neuropathology**

a. **Brain weights:** There were no treatment-related effects on absolute or relative brain weights in male or female offspring at postnatal days 11, 21, or 63-67. Mean brain weight data (for those animals selected for neuropathology) are presented in Table 15.

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TABLE 15. Mean (±SD) Brain Weight Data in Perfused Offspring [a]				
Parameter	Dose (mg/kg/day)			
	0	5	50	150
Males				
PND 11 (N = 11-12)				
Terminal body weight (g)	22.4±5.6	22.6±3.5	24.3±4.3	24.0±3.4
Brain weight (g)	1.110±0.154	1.118±0.124	1.182±0.146	1.141±0.109
Brain-to-body weight ratio	5.147±0.832	4.983±0.381	4.945±0.582	4.791±0.342
PND 21 (N = 9-10)				
Terminal body weight (g)	52.9±5.7	57.3±3.9	56.2±4.4	53.0±5.3
Brain weight (g)	1.396±0.114	1.398±0.134	1.407±0.064	1.409±0.056
Brain-to-body weight ratio	2.669±0.366	2.445±0.224	2.515±0.163	2.682±0.298
PND 63-67 (Termination) (N = 10)				
Terminal body weight (g)	413.2±26.2	393.2±49.0	415.6±28.4	404.1±27.5
Brain weight (g)	2.044±0.160	1.994±0.152	2.098±0.079	2.031±0.157
Brain-to-body weight ratio	0.496±0.043	0.512±0.048	0.507±0.034	0.504±0.040
Females				
PND 11 (N = 11-12)				
Terminal body weight (g)	23.5±3.6	24.9±3.0	23.1±4.2	21.3±5.2
Brain weight (g)	1.146±0.088	1.190±0.096	1.148±0.154	1.074±0.134
Brain-to-body weight ratio	4.931±0.521	4.805±0.299	5.041±0.473	5.251±1.016
PND 21 (N = 10-11)				
Terminal body weight (g)	51.7±6.5	53.0±6.0	51.7±4.6	51.6±6.4
Brain weight (g)	1.334±0.129	1.364±0.112	1.362±0.083	1.381±0.106
Brain-to-body weight ratio	2.606±0.320	2.587±0.239	2.651±0.258	2.700±0.269
PND 63-67 (Termination) (N = 10)				
Terminal body weight (g)	248.3±18.3	256.1±23.6	249.2±12.0	252.4±18.6
Brain weight (g)	1.904±0.103	1.878±0.132	1.935±0.121	1.879±0.108
Brain-to-body weight ratio	0.769±0.050	0.736±0.045	0.777±0.052	0.749±0.081

a Data obtained from pages Tables 53, 55 & 63, pages 142, 145, & 156. MRID 45646401.

Brain weights were also evaluated for non-perfused animals sacrificed at day 65 (n=60/60, 59/56, 52/50, and 56/58 [M/F] for the control, 5 mg/kg/day, 50 mg/kg/day, and 150 mg/kg/day groups, respectively). Consistent with the results for neuropathology animals, brain weights were similar across all treatment groups in these additional animals.

b. Neuropathology

1. **Macroscopic examination:** No treatment-related effects were reported for male or female offspring at postnatal days 11, 21, or 63-67.

2. **Microscopic examination:** No significant treatment-related effects were noted at histopathological examination of tissues from offspring terminated on postnatal days 21 or 63-67. For PND 21 offspring, changes in the kidney (Table 16) were considered incidental to

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treatment since incidence and severity were similar in control and high-dose animals. Cortical tubular dilatation was considered an artifact of the perfusion procedure.

No significant treatment-related effects were noted on postnatal day 63-67. The study author considered changes in the kidney to be incidental to treatment; however, the incidence of findings in the PND 63-67 males at 150 mg/kg/day (Table 16a) is suggestive of a treatment-related response. Cortical tubular dilatation was again considered an artifact of the perfusion procedure. Minimal or slight degenerative changes in peripheral nerves were noted in both control and high-dose animals with similar incidences and severity, with the exception of a higher incidence of effects in the sciatic nerve of controls (Table 16b).

TABLE 16a. Incidence of Kidney Lesions for Perfused Offspring [a]				
	Males		Females	
Observation	0 mg/kg/day	150 mg/kg/day	0 mg/kg/day	150 mg/kg/day
	PND 21			
No. examined	7/11	4/11	6/13	8/11
Cortical tubular dilatation	7	4	6	7
Cortical cyst(s)	2	1	4	2
Hydronephrosis	5	3	5	8
Papilla - dilated tubules	1	0	0	0
Medullary cyst(s)	0	0	0	1
	PND 63-67			
No. examined	2/10	7/10	0/10	2/10
Hydronephrosis	2	5	0	1
Cortical tubular dilatation	2	7	0	2
Papilla - dilated tubules	0	1	0	0
Medullary cyst(s)	0	1	0	1
Mineralization, papilla	0	1	0	0
Cortical tubular vacuolation	2	7	0	2
Cortical tubular basophilia	0	2	0	0
Cortical scarring	0	2	0	1
Cortical cyst(s)	0	2	0	1
Tubular casts	0	1	0	0
Cortical tubules with hyaline droplets	0	1	0	0

a   Data obtained from Tables 59 & 68, pages 150 & 166. MRID 45646401.

TABLE 16b. Incidence of Peripheral Nerve Lesions for Perfused Offspring (PND 63-67) [a]				
	Males		Females	
Observation	0 mg/kg/day	150 mg/kg/day	0 mg/kg/day	150 mg/kg/day
No. examined	10/10	10/10	10/10	10/10
Eyes - retinal rosettes/folds	0	0	1	1
Sciatic nerve - degenerate fibers	8	3	4	3
Tibial nerve - calf - degenerate fibers	3	5	3	4

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TABLE 16b. Incidence of Peripheral Nerve Lesions for Perfused Offspring (PND 63-67) [a]				
	Males		Females	
Observation	0 mg/kg/day	150 mg/kg/day	0 mg/kg/day	150 mg/kg/day
Tibial nerve - knee - degenerate fibers	3	3	2	2

a Data obtained from Table 68. page 165, MRID 45646401.

There were no differences in brain length or width in males o, females on postnatal days 21 or 63-67. Data are summarized in Table 17.

TABLE 17. Mean (±SD) Brain Length and Width Data for Offspring [a]				
	Dose (mg/kg/day)			
Parameter	0	5	50	150
Males				
PND 21				
Brain length (mm)	18.0±0.5	18.2±0.6	18.3±0.3	18.0±0.3
Brain width (mm)	14.6±0.4	14.8±0.1	14.7±0.3	14.6±0.5
PND 63-67 (Termination)				
Brain length (mm)	21.8±0.6	21.5±0.4	21.6±0.5	21.4±0.5
Brain width (mm)	15.3±0.3	15.5±0.4	15.6±0.5	15.3±0.5
Females				
PND 21				
Brain length (mm)	17.5±0.9	17.9±0.6	18.1±0.4	17.9±0.4
Brain width (mm)	14.7±0.4	14.5±0.3	14.5±0.5	14.6±0.4
PND 63-67 (Termination)				
Brain length (mm)	20.9±0.4	21.0±0.5	21.0±0.4	21.0±0.6
Brain width (mm)	15.4±0.6	15.3±0.4	15.1±0.6	14.9±0.6

a Data obtained from pages Tables 56 & 64. pages 146 & 157. MRID 45646401.
N = 9-10/sex/dose

Morphometric data for males or females on postnatal days 21 and 63-67 revealed an increase in the mean corpus callosum measurement for 150 mg/kg/day males and females on PND 63-67 (Table 18a). This finding was statistically significant for 150 mg/kg/day females but not for males. although the magnitude of the change was equivalent for both sexes. The individual data (Table 18b) demonstrate a shift in the measures for both sexes and supports the interpretation of this finding as being treatment-related rather than incidental. The brains of the 5 and 50 mg/kg/day offspring were not evaluated histopathologically. In accordance with guideline recommendations. a morphometric evaluation of the corpus callosum for the low- and mid-dose adult offspring is required by the Agency to establish the dose-response nature of this finding.

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TABLE 18a. Mean (±SD) Morphometric Data for Offspring [a]		
Parameter	**Dose (mg/kg/day)**	
	0	**150**
Males		
PND 21		
Neocortex (mm)	1.85±0.18	1.84±0.14
Hippocampus (mm)	1.68±0.17	1.72±0.16
Corpus Callosum (mm)	0.18±0.06	0.15±0.02
Cerebellum (mm)	0.78±0.11	0.76±0.07
PND 63-67 (Termination)		
Neocortex (mm)	1.94±0.14	1.89±0.16
Hippocampus (mm)	2.03±0.15	2.03±0.19
Corpus Callosum (mm)	0.29±0.07	0.36±0.11
Cerebellum (mm)	0.82±0.05	0.86±0.09
Females		
PND 21		
Neocortex (mm)	1.83±0.13	1.91±0.12
Hippocampus (mm)	1.74±0.11	1.72±0.22
Corpus Callosum (mm)	0.19±0.09	0.21±0.05
Cerebellum (mm)	0.75±0.07	0.81±0.07
PND 63-67 (Termination)		
Neocortex (mm)	1.95±0.09	1.86±0.12
Hippocampus (mm)	1.97±0.11	2.05±0.10
Corpus Callosum (mm)	0.29±0.06	0.36*±0.08
Cerebellum (mm)	0.87±0.06	0.89±0.11

a Data obtained from pages Tables 58 & 67. pages 149 & 164. MRID 45646401.
N = 8-10/sex/dose. *p<0.05

TABLE 18b. Distribution of Individual Corpus Callosum Measures for PND 63-67 Offspring [a]				
Corpus Callosum Measurement (mm)	Males		Females	
	0 mg/kg/day	150 mg/kg/day	0 mg/kg/day	150 mg/kg/day
0.20-0.24	5	2	4	1
0.25-0.29	1	1	1	1
0.30-0.34	2	1	3	2
0.35-0.39	1	3	1	2
0.40-0.44	1	1	1	4
0.45-0.49	0	1	0	0
≥0.50	0	1	0	0

a Data obtained from Appendix 64, pages 1213 & 1214, MRID 45646401. ·
N = 10/sex/dose

III. ·DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that treatment of the dams between GD6 and PND 10 with malathion at doses of 5, 50. or 150 mg/kg/day had no effects on clinical condition. survival, body weight gain, food consumption during gestation or lactation, gestation length or macroscopic necropsy findings. The authors attributed the increase in post-dosing salivation to distaste of the formulation, not to an effect of treatment.

The investigators concluded that 50 mg/kg/day was the NOAEL for morphological and functional development of the nervous system in the CD rat. following maternal exposure from implantation until mid-lactation, followed by direct treatment of the offspring from PND 11 to 21. The LOAEL established by the investigators was 150 mg/kg/day. based upon 1) adverse post-dosing signs on PND 17-19, including one or more of the following: whole body tremors, underactivity, prostrate, eyelids partially closed and abnormal gait; and 2) transient delayed surface righting reflex in PND 11 females.

B. **REVIEWER COMMENTS:** There were no treatment-related maternal deaths before scheduled termination. Clinical signs were limited to transient post-dosing salivation (5/24 control. 4/24 at 5 mg/kg/day. 3/24 at 50 mg/kg/day, and 20/24 at 150 mg/kg/day). Although the study authors attributed the increase in post-dosing salivation to distaste of the formulation. it is the opinion of the Agency reviewers that this effect is treatment-related. There were no other treatment-related effects on cholinergic signs. and there were no effects on maternal body weight. food consumption. or reproductive indices. Based upon the parameters assessed in this study. the **maternal LOAEL for malathion in rats is 150 mg/kg/day, based on an increased incidence of post-dosing salivation.** The **maternal NOAEL is 50 mg/kg/day**. It is noted that in a companion comparative cholinesterase study with malathion (MRID 45566201). approximately 20% RBC cholinesterase inhibition was demonstrated at 50 mg/kg/day in maternal animals dosed by gavage from GD 6-20, and in young adult rats that were dosed by gavage for 11 consecutive days. At 150 mg/kg/day, 51%

RBC cholinesterase inhibition was observed in maternal animals, and 43-48% RBC cholinesterase inhibition was observed in young adults.

For offspring, there were no differences among treatment groups with respect to pup survival, body weight or food consumption, day of sexual maturation, learning and memory evaluations, or brain weights and external brain measurements (length and width).

There were no clinical signs noted prior to direct treatment of offspring on PND 11. Four offspring (all from one litter) in the 150 mg/kg/day group exhibited whole body tremors and hypoactivity after dosing on PND 17 and 18. Two of these pups also exhibited prostrate posture and partially closed eyelids on PND 17 and another of these pups showed abnormal gait on PND 19. At 50 mg/kg/day, observations were limited to transient post-dosing salivation in one pup on PND 11. At 5 mg/kg/day, observations were limited to transient post-dosing salivation in one pup on PND 9. No other treatment-related clinical signs were reported in pups.

The mean surface righting score for PND 11 female pups at 150 mg/kg/day was increased (1.6) as compared to control females (1.0). Other treatment-related findings included a slightly flattened gait in 3/10 mid-dose (50 mg/kg/day) males and 6/10 high-dose (150 mg/kg/day) males on PND 60. No other treatment-related effects were noted in arena observations or functional observations of hand-held animals.

On PND 17 and 22, decreased rearing and cage floor activity were noted for treated females compared to controls. A dose response was observed on both days, and the effect reached statistical significance ($p<0.05$ or 0.01) for cage floor activity at 50 and 150 mg/kg/day at PND 22. The decreased cage floor activity noted for PND 17 and 22 females at 50 and 150 mg/kg/day is supported by the decreased rearing activity at the same ages and doses, and is considered an effect of treatment at those doses. No other significant motor activity effects were noted for females.

Mean startle amplitudes for treated offspring were higher than controls on PND 23/24 in all five testing blocks. Mean amplitudes reached statistical significance for PND 23/24 females at 5 mg/kg/day (block 5), 50 mg/kg/day (blocks 4 and 5), and 150 mg/kg/day (block 5). Although a clear dose-response relationship was not established, the magnitude of the response was generally equivalent across all treated groups, and the overall consistency of the effect throughout all testing sessions and at all treatment levels was considered particularly noteworthy. It was noted that the control values for the PND 23/24 males and females were quite similar to the control (and treated group) values reported for another developmental neurotoxicity study with dimethoate that was conducted in the same laboratory (MRID 45529703), demonstrating that the increases in peak amplitude in the treated groups were not attributable to aberrant control data. Therefore, the increased startle amplitude in PND 23/24 males and females was considered treatment-related at all dose levels. At PND 60/61, group mean startle amplitudes for treated females were lower than controls, especially during the first two blocks. No effects were noted for male offspring at PND 60/61. The change in mean peak amplitude across testing sessions for PND 60/61 males at 150 mg/kg/day and for females at all treatment levels was notably less than the control values, indicating a treatment-related

effect on habituation. No consistent differences in the degree of pre-pulse inhibition were seen in either sex at any dose.

Morphometric evaluation of offspring brains revealed an apparent treatment-related increase in the corpus callosum measurement for both PND 63-67 males and females ($p < 0.05$) at 150 mg/kg/day. The brains of the 5 and 50 mg/kg/day offspring were not evaluated histopathologically. In accordance with guideline recommendations, a morphometric evaluation of the corpus callosuŗ, for the low- and mid-dose adult offspring is required by the Agency to establish the dose-response nature of this finding.

In summary, the **offspring NOAEL is <5 mg/g/day**. The **offspring LOAEL is 5 mg/kg/day, based upon increased auditory startle reflex peak amplitude in PND 23/24 male and female offspring**. At higher doses the following additional findings were observed: At 50 mg/kg/day, there was an increased incidence of slightly flattened gait in PND 60 males, and motor activity counts (rearing and ambulatory) were decreased in female pups at PND 17 and 22. At 150 mg/kg/day, additional treatment-related findings included post-dosing clinical observations on PND 17 and 18 (increased incidences of whole body tremors, hypoactivity, prostrate posture, partially closed eyelids, and/or abnormal gait), delayed surface righting reflex in PND 11 female pups, increased incidences of slightly flattened gait in PND 60 males, and increased thickness of the corpus callosum in PND 63-67 males and females.

Comparative Cholinesterase Study: In the companion cholinesterase inhibition study (MRID 45566201), 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 on RBC cholinesterase 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 on RBC cholinesterase 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, brain cholinesterase was inhibited at 150 mg/kg/day following an acute dose (44-48%) in PND 11 pups or after 11 repeated doses (16%) in PND 21 pups. Based upon the results of the cholinesterase study, it is evident that all behavioral and neuropathological effects of treatment observed in the dams and offspring in the developmental neurotoxicity study occurred at doses at which cholinesterase was, or had been, inhibited.

Latent effects: It is noted that some of the treatment-related findings in offspring were observed at PND 60, that is, 39 days after the last dose (on PND 21). The comparative cholinesterase study (MRID 45566201) demonstrates that cholinesterase levels had returned to baseline by that time. Yet slightly flattened gait was observed in PND 60 males at 50 and 150 mg/kg/day, decreased habituation in auditory startle response was noted at 150 mg/kg/day for PND 60/61 males and at all treatment levels for PND 60/61 females, and increased corpus callosum measures were observed in the brain of both sexes in the PND 63-67 terminal studies. Since the time course of these findings was not studied, it is unknown whether these adverse effects, which appear to have a delayed onset, will be transient or permanent.

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Adequacy of dosing: Dose levels selected for this study were based upon the severe toxicity to pups (mortality) following gavage dosing that initiated at PND 11 in a dose range-finding developmental neurotoxicity study (MRID 45627001). This ensured the survival of pups once direct dosing commenced in the definitive developmental neurotoxicity study. In a companion comparative cholinesterase study in rats (MRID 45566201), gavage dosing of dams from GD 6-20 resulted in maternal and fetal RBC cholinesterase inhibition (19 and 51% in dams, 11 and 19% in fetuses, at 50 and 150 mg/kg/day, respectively) and a high incidence of post-dosing salivation at 150 mg/kg/day. In that study, there was no evidence of cholinesterase inhibition in PND 4 pups. The apparent recovery in cholinesterase activity by PND 4 under these study conditions could have been 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. Thus, while neurological development occurring from PND 11-21 was adequately assessed, there is uncertainty regarding the adequacy of the assessment of potential alteration in significant stages of neurological development that occur from birth through PND 10 in the rat.

Although maternal doses might have been increased during gestation and/or early lactation in order to attempt to increase the dose to the pups during those life stages, such procedures would have made the interpretation of the data more difficult, and might have additionally compromised the study results. The study was conducted according to guideline recommendations, and the study identified maternal and offspring NOAELs and/or LOAELs. It is recognized that there are limitations of the study design, most significantly the need to rely upon maternal dosing for 1) *in utero* fetal exposure and 2) early lactation exposure to pups, and the resulting inability to quantify exposure to the developing individual during these stages. However, these limitations are generic issues, and do not affect the acceptability of this study. Overall, the dose levels used in this study and the dosing methodology were considered to be adequate and acceptable. (Note: this conclusion represents the opinion of one of the authors of this DER, and was supported by peer review of the Hazard Identification Assessment Review Committee [TXR 0050804] and the Developmental Neurotoxicity Protocol Review Committee [TXR 0051035]. A dissenting opinion, from the second DER author, is presented in Appendix 2 to this DER.)

This study is classified **Acceptable Guideline** and satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6).

C. **STUDY DEFICIENCIES:**

Although an apparent treatment-related increase in corpus callosum measurements was noted in PND 63-67 males and females at 150 mg/kg/day, the brains of the 5 and 50 mg/kg/day offspring were not evaluated histopathologically. In accordance with guideline recommendations (OPPTS 870.6300), a morphometric evaluation of the corpus callosum for the low- and mid-dose adult offspring is required by the Agency to establish the dose-response nature of this finding.

The coefficients of variation for motor activity measurements (rearing and ambulatory) in pre-weaning rats were excessive, exceeding 200% for some time points. This made changes that

were seen in these measures more difficult to interpret, due to a lack of statistical significance. One approach that might have helped with analysis of these data would be to make use of repeated measures analyses of variance (see e.g., Tamura, R.N. and J. Buelke-Sam (1992) The use of repeated measures analysis in developmental neurotoxicity studies. *Neurotoxicol. Teratol.* 14:205-210.)

The following deficiencies were noted in positive control data previously submitted to the Agency in MRIDs .5308301 and 45308302 (see Appendix A). 1) The submitted data did not address functional observational battery data or histopathology (including morphometrics) following administration of positive control substances. 2) Differences were noted between the procedures used in auditory startle and motor activity testing for the positive control and malathion studies (see Table A1-3). 3) Motor activity data were highly variable. 4) Morris maze results showed a strong effect, but the dose may have been too high. Therefore, it may be questionable as to whether the effect is specific to learning/memory (large number of sector entries indicate animals were possibly just swimming around and around the pool – increased activity/thigmotaxis may be caused by scopolamine at high doses). Nevertheless, in spite of any deficiencies, the positive control data were found to provide some level of confidence in the ability of the performing laboratory to assess specific neurobehavioral parameters in immature rats.

Appendix 1 - DNT Positive Control Data

Positive control data were previously submitted to EPA to demonstrate the proficiency of the performing laboratory (Huntingdon Life Sciences Ltd., Eye Suffolk, IP23 7PX England) in conducting developmental neurotoxicity studies. Summaries of these studies are presented in Appendices A1 through A3.

Citations:

Myers, D.P. (2000). 1) Assessment of the effects of amphetamine or chlorpromazine on the motor activity of young rats – positive control study, and 2) Examination of brains from untreated 11-day old rats – Background control data. Huntingdon Life Sciences Ltd., Eye Suffolk, IP23 7 PX, England. HLS058/983891, November 30, 2000. MRID 45308301. Unpublished.

Myers, D.P. (2000). Assessment of the effect of scopolamine on auditory startle response and Morris water maze learning in young rats. Positive Control Study. Huntingdon Life Sciences Ltd., Eye Suffolk, IP23 7 PX, England. HLS059/002438, December 4, 2000. MRID 45308302. Unpublished.

Overall assessment of the adequacy of the positive control submission: The submitted positive control studies included full reports with individual and summary data and procedural information. Documentation of a Quality Assurance review was provided. The positive control studies were conducted approximately 3 years prior to the malathion DNT study, using the same strain of rat. The submitted studies assessed a number of neurobehavioral tests required in a guideline DNT study, including auditory startle habituation and pre-pulse inhibition, motor activity, and cognitive function. Evaluations were conducted in age-appropriate animals. Alterations in selected neurobehavioral parameters were reported following administration of positive control materials. The following deficiencies were noted: 1) The submitted data did not address functional observational battery data or histopathology (including morphometrics) following administration of positive control substances. 2) Differences were noted between the procedures used in auditory startle and Morris maze (learning and memory) testing for the positive control and malathion studies (see Table A1-3). 3) Motor activity data (including control data) were highly variable, which limits sensitivity. 4) Scopolamine is not an appropriate positive control substance for auditory startle response testing; the submitted data are not considered to be adequate to demonstrate proficiency in auditory startle testing. 5) Morris maze results showed a strong effect, but only at a dose known to cause increases in motor activity levels; therefore, the specificity of the results to learning/memory deficits cannot be verified. This issue could be resolved by generating a dose/response curve starting with lower doses.

Appendix 1
POSITIVE CONTROL DATA SUMMARY
Table A1-1

Positive Control Chemical	Amphetamine (D-Amphetamine Sulfate) [0.9% saline control]							
Date of Positive Control Data: May, 1998								Citation: Myers, D.P. (2000) 1) Assessment of the effects of amphetamine or chlorpromazine on the motor activity of young rats – positive control study, and 2) Examination of brains from Untreated 11-day old rats. Background control data. Huntingdon Life Sciences
Species/Strain: Sprague Dawley (Charles River)								Ltd., Eye Suffolk, IP23 7 PX, England. HLS058/983891, November 30, 2000. MRID 45308301. Unpublished.

Methods	Method Codes	Data Present?	Age Relevant?	Ages (days)	Dose Levels	Sexes (m/f)	Group Size	Comments/Effects
Dev Landmarks								
FOB								
Motor Activity	Infrared - low and high beams	yes	yes	PND 13, 17, 22	2.0 mg/kg; oral gavage	both	10/sex	Rats placed in activity monitor at 30-min. post-dose; 60-min. session length, 6-min subsessions
Startle								
Learn/Memory								
Std Histopath	control only	yes		PND 11	control	both	15/sex	
Morphometrics	control only	yes		PND 11	control	both	15/sex	Brain length, width, weight, histopathology, and morphometry of major layers in neocortex, hippocampus, corpus callosum
Overall (subjective assessment of data quality)	Data report was adequate (individual data, methods, etc). Results: Data indicated large (statistically significant) increase in motor activity for all days evaluated. Increases occurred throughout the 1-hr recording period and were evident in low beam scores at all three test ages and in high beam scores on PND 17 and 22. Deficiencies: 1) Variance was very high. 2) Morphometric evaluation was control data only, with only one age evaluated; these data are useful as historical control only							

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Appendix 1

POSITIVE CONTROL DATA SUMMARY
Table A1-2

Positive Control Chemical	Chlorpromazine (Chlorpromazine hydrochloride) - saline control	Citation: Myers, D.P. (2000) 1) Assessment of the effects of amphetamine or chlorpromazine on the motor activity of young rats – positive control study, and 2) Examination of brains from Untreated 11-day old rats – Background control data. Huntingdon Life Sciences Ltd., Eye Suffolk, IP23 7 PX, England. HLS058/983891. November 30, 2000. MRID 45308301. Unpublished.
Date of Positive Control Data: May, 1998		
Is Species/Strain the same as in Main Study?: Sprague Dawley (Charles River)		

Methods	Method Codes	Data Present?	Age Relevant?	Ages (days)	Dose Levels	Sexes (m/f)	Group Size	Comments/Effects
Dev Landmarks								
FOB								
Motor Activity	Infrared - low and high beams	yes	yes	PND 13, 17, 22	10 mg/kg (oral gav.)	Both	10/sex	Rats placed in activity monitor at 30-min. post-dose; 60-min session with 6-min subsessions
Startle								
Learn/Memory								
Std Histopath								
Morphometrics								

Overall (subjective assessment of data quality)	Data report was adequate (individual data, methods, etc). Results: Small but consistent decrease in motor activity on all testing days, throughout the 1-hr test period (low beams at all ages, high beams at PND 17 and 22); not always statistically significant Deficiencies: High variance in control is as much a problem as is the small decrease with chlorpromazine.

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POSITIVE CONTROL DATA SUMMARY
Table A1-3

Positive Control Chemical	Scopolamine	Citation: Myers, D.P. (2000). Assessment of the effect of scopolamine on auditory startle response and Morris water maze learning in young rats. Positive Control Study. Huntingdon Life Sciences Ltd., Eye Suffolk, IP23 7 PX, England. HLS059/002438, December 4, 2000. MRID 45308302. Unpublished.
Date of Positive Control Data: May, 1998		
Species/Strain: Sprague Dawley (Charles River)		

Methods	Method Codes	Data Present?	Age Relevant?	Ages (days)	Dose Levels	Sexes (m/f)	Group Size	Comments/Effects
Dev Landmarks								
FOB								
Motor Activity								
Startle	San Diego Instruments	yes	yes	PND 26, 63	1.5 mg/kg (i.p.) in 5 ml/kg	both	8-10/sex	5 blocks of 6 trials at day 26; 5 blocks of 10 trials at d. 63; pre-pulse evaluated on d. 26 only
Learn/Memory	Morris water maze	yes	yes	PND 28, 63	same	both	10/sex	3 trials/day for 3 consecutive days, otherwise same (main study used 4 days)
Std Histopath								
Morphometrics								

| Overall (subjective assessment of data quality) | Data report was adequate (individual data, methods, etc); however, statistical methods were not clearly defined. Results/deficiencies:

Learning/memory: Morris maze results show a strong effect, but scopolamine is known to cause increases in motor activity at similar doses (Crofton KM et al., Interlaboratory comparison of motor activity experiments: Implications for neurotoxicological assessments. Neurotoxicol. Teratol. 1991; 13:599-609; Crofton, KM and MacPhail RC. Reliability of motor activity assessments. In: Measuring Movement and Locomotion: From Invertebrates to Humans, K-P Ossenkopp, M. Kavaliers, and P.R. Sanberg, (Eds), R.G. Landes Company, 1996), and deficits seen in the submitted study could be due to increased activity. This issue could be resolved by generating a dose/response curve starting with lower doses.
Auditory startle: Not all the controls showed habituation on day 26; habituation did not appear to be altered by treatment. Scopolamine is not an appropriate PC substance for auditory startle testing. Literature references report either no effect of scopolamine on startle amplitude (Jones, CK and Shannon HE. Muscarinic cholinergic modulation of prepulse inhibition of the acoustic startle reflex.. J Pharmacol Exp Ther, 2000 Sep;294(3):1017-23.) or slight increases (Sipos, ML, Burchnell, V, Galbicka, G. Effects of selected anticholinergics on acoustic startle response in rats. J Appl Toxicol. 2001 Dec;21 Suppl 1:S95-101), while this study reports decreased amplitude at PND 63. Baseline levels were quite variable; findings from pre-pulse inhibition appeared much stronger. |
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Appendix 2 - Dissenting Opinion to Adequacy of Dose Levels

MEMORANDUM

From:	Brian Dementi
Date:	06/20/02 04:03 PM
To:	Marcia Mulkey/DC/USEPA/US@EPA
cc:	John Carley/DC/USEPA/US@EPA, Elizabeth Doyle/DC/USEPA/US@EPA, John Hirzy/DC/USEPA/US@EPA
Subject:	HIARC Dissenting Opinion/Inadequate High Dose/Malathion DNT Study

Dear Ms Mulkey

As covered in the December 14, 2000 (TXR014413) and April 19, 2001 (TXR014544) reviews (Dementi) of 6(a)(2) data submitted on the preliminary cholinesterase and DNT studies, an MTD should have been employed in the definitive studies. Severe toxicity was observed in dams at 750 mg/kg/day and in offspring at 200 mg/kg/day in preliminary studies. The sponsor chose a high dose level of 150 mg/kg/day for the definitive studies in order to preclude severe toxicity to offspring in the direct dosing of the offspring add-on phase to the Guideline DNT study. If only the Guideline DNT study had been pursued, which does not include direct dosing of offspring, it is anticipated the sponsor, by analogy, would have backed off the dosing of dams to either 700 mg/kg/day (50 dosing units lower than the high dose), or to 560 mg/kg/day (3/4 of the high dose) as was done to protect offspring from severe toxicity. Absent any further testing of adults above 150 mg/kg/day, one must conclude that predictably this was not an MTD or otherwise adequate high dose for the study. Furthermore, the definitive study being conducted at doses no higher than 150 mg/kg/day leaves unaddressed the magnitude of offspring susceptibility *for this frank clinical toxicity (moribundity/death)* already evident as being on the order of magnitude of 200 mg/kg/day (offspring) versus 750 mg/kg/day (dam) in the range-finding studies.

A possible solution to the dosing problem for both dams and offspring in the definitive study that would have likely addressed this aspect of the susceptibility question would have been to conduct all but the direct dosing of offspring aspect at 700 (560) mg/kg/day and the offspring testing at 150 mg/kg/day.

This evident contrast of toxicity between dam and offspring, and the need for adequate high dose testing of dams is well presented in the two reviews of 6(a)(2) cited above, as well as in the 6/13/01 email of Brian Dementi to SRRD's Carmelita White responding to Cheminova's rationale for dose selection (Attachment). [EDITORIAL NOTE: E-MAIL ATTACHMENT IS NOT APPENDED BUT CAN BE FOUND IN HIARC REPORT, TXR 0050804, DATED JUNE 13, 2002.]

Therefore, the dosing of dams in the DNT study did not test at an adequately high dose level and for this reason is arguably *unacceptable*. To the extent that for whatever reason the study is considered *acceptable*, it must be conceded up front, that for the very reason of inadequately low, high-dose selection for the study, a *susceptibility* for the severe toxicity (moribundity/death) aspect, on the order of magnitude of 700/150 (4.7X) or 560/150 (3.7X), or about 4X exists.

Furthermore, if the study is determined to be *unacceptable*, a standard 3X FQPA factor for a missing study is thus inadequate. I should note that should a study conducted at doses no higher than 150 mg/kg/day as administered orally to dams yield no clear findings in either dams or offspring (at a dose level below the MTD for dams), the study would be inconclusive. Furthermore, even in the event that modest effects are observed at this low dose level, the study may not prove as definitive as it might when testing is done at the MTD or otherwise proper dose level as prescribed for dams in the Guideline DNT study.

These arguments were presented to the May 20 HIARC. The committee nonetheless concluded that the high dose level was adequate, as explained under Topic B (p. 70) of the June 13, 2002 HIARC report (TXR# 0050804): "The HIARC concurred with the conclusion of the DNT PRC, that the DNT study was acceptable and that the doses were adequate. The primary evidence supporting this conclusion included: 1) offspring effects were noted at the lowest dose tested in this study (5 mg/kg/day), 2) range-finding data indicated that 200 mg/kg/day would be lethal to young pups that were being dosed by gavage (initiating at PND 11), 3) maternal toxicity (post-dosing salivation, a cholinergic response, in the DNT, and cholinesterase inhibition in the companion comparative cholinesterase study) was observed at the high-dose level (150 mg/kg/day). The absence of a NOAEL for offspring in the study was not felt to compromise the adequacy of the study for use in risk assessment and was not considered a fatal flaw. It was noted that the study was conducted according to the guideline (OPPTS 870.6300) and the specifications of the Data-Call-In for DNT studies on the organophosphate pesticides with tolerances."

I heartily disagree with the conclusions of the DNT PRC and with HIARC, and believe neither committe has risen to the occasion of acknowledging that the high dose level of 150 mg/kg/day is inadequate as a high dose for use in the DNT guideline testing. Reasons 1 and 2 offered by HIARC are irrelevant to the question of the adequacy of the high dose. I do not agree with the claim in item 3 that the study was conducted according to the DNT guideline (OPPTS 870.6300) insofar as the guideline speaks to high dose selection. That guideline reads:

"(3) Dose levels and dose selection. (i) At least three dose levels of the test substance plus a control group (vehicle control, if a vehicle is used) should be used.

(ii) If the test substance has been shown to be developmentally toxic either in a standard developmental toxicity study or in a pilot study, the highest dose level should be the maximum dose which will not induce in utero or neonatal death or malformations sufficient to preclude a meaningful evaluation of neurotoxicity. *Comment: according to this criterion, the highest dose could be the highest dose employed in the Guideline rat developmental toxicity study, which was 800 mg/kg/day, at which dose there were dam bodyweight decreases commensurate with an acceptable high dose, with no developmental effects, i.e. for developmental toxicity, LOAEL > 800 mg/kg/day. However, given the surprising findings at 750 mg/kg in the range-finding DNT study, and this dose would be too high, it is nevertheless likely an acceptable high dose would have been well above 150 mg/kg/day in dams*

(iii) If a standard developmental toxicity study has not been conducted, the highest dose level, unless limited by the physicochemical nature or biological properties of the substance, should

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induce some overt maternal toxicity, but should not result in a reduction in weight gain exceeding 20 percent during gestation and lactation. *Comment: salivation was the sole clinical sign at 150 mg/kg/day, which the study author attributed to bad taste of the material, and which I have affirmed, and further argued constitutes inadequate evidence of cholinergic toxicity to identify an MTD or otherwise sufficiently high dose level for the study's highest dose. The presence of but the one cholinergic sign (salivation) from among a generally recognized group of such signs, taken in concert with modest erythrocyte cholinestrease inhibition (particularly the latter effect) qualify as an effec. level at 150 mg/kg/day, but in terms of magnitude of effect sought under the protocol, this response is quite deficient for the high dose group. I view as questionable whether it even rises to the level prescribed for the intermediated dose. There were no dosing related alterations in maternal body weight at any dose level up to and including the high dose of 150 mg/kg/day.*

(iv) The lowest dose should not produce any grossly observable evidence of either maternal or developmental neurotoxicity.

(v) The intermediate dose should be equally spaced between the highest and lowest doses used." (pp. 2-3)

According to this definition, 150 mg/kg/day would not satisfy as an adequately high dose level, or an MTD, which in turn is recognized as important to the full exploitation of the spectrum of toxicity in the developing individual. Indeed, I again question whether 150 mg/kg/day is adequately high to serve as an intermediate dose, let alone the high dose called for in the protocol. If 150 mg/kg/day is or approaches an MTD, as it should, then it serves to underscore the dramatic variability in clinical toxicity in the malathion data base as compared with high dose levels in the variety of other studies.

It would be helpful to me if HIARC confirmed that had the study been performed simply as a guideline DNT study, absent the direct dosing of pups add on component, higher dosing than 150 mg/kg/day would have been pursued. However, in any case, regardless of the opinions of the DNT PRC and HIARC, I must maintain the legitimacy of my reasoning.

Inadequate dosing at the higher dose levels might have precluded the identification of behavioral effects that could suggest more serious effects in offspring than those identified, or that a 3X factor to correct for the absence of a NOAEL in offspring is inadequate. One cannot *reliably* anticipate what effects might have been observed had the DNT protocol been followed, which is why studies are conduced, and in a prescribed manner. Unanticipated effects are often observed. I would suggest that this deficiency might be better remedied by applying an uncertainty factor, rather than denying the flaw that plainly exists. It is surprising that the possibility was not recognized at the outset, that pups might not endure a dose level on the order of an MTD in the adult.

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

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
057701	45646401	DNT	rat	GD 6-LD 10	oral	gavage	N/A	5, 50, 150 Vehicle = corn oil	50	150	Increased incidence of post-dosing salivation	Maternal
057701	45646401	DNT	rat	PND 11-21	oral	gavage	N/A	5, 50, 150 Vehicle = corn oil	<5	5	Increased auditory startle reflex peak amplitude in PND 23/24 ♂/♀; decreased habituation in PND 60/61 ♀	Offspring

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