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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: **September 21, 2005**

MEMORANDUM

TXR No.: **0052433**

SUBJECT: Phorate - Evaluation of a Developmental Neurotoxicity

FROM: William Greear, M.P.H., D.A.B.T., Toxicologist
Registration Action Branch 1
Health Effects Division (7509C)

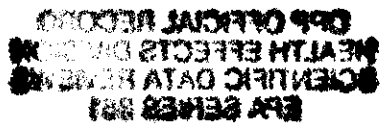
THROUGH: Pv Shah, Ph.D., Branch Senior Scientist
Registration Action Branch 1
Health Effects Division (7509C)

TO: Susan Lewis, Product Manager
RM-# 51
Special Review and Reregistration Division (7505C)

DP Barcode: 300057 **PC Code:** 057201

HED Conclusions: The developmental neurotoxicity study is classified as Acceptable/Non Guideline.

Action Requested: Review of a developmental neurotoxicity study in rats submitted by Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany.



Results of the Submitted Studies:

1. **CITATION:** Kaufmann, W., S. Schneider, K. Deckardt, *et al* (2004) BAS 225 I (phorate) - Developmental Neurotoxicity Study in Wistar Rats Oral Administration to the Dams and Pups (Gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Project I.D. 66R0091/02039; February 23, 2004. MRID 46214401. Unpublished

Kaufmann, W., S. Schneider, K. Deckardt, *et al* (2004) BAS 225 I (phorate) - Developmental Neurotoxicity Study in Wistar Rats Oral Administration to the Dams and Pups (Gavage) (Supplemental Report - Additional Dose Level). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Project I.D. 66R0091/02052; February 23, 2004. MRID 46214402. Unpublished

EXECUTIVE SUMMARY:

NOTE: This Data Evaluation Record contains the review of two developmental neurotoxicity studies. The initial study that tested three dose level (MRID 46214401) and a supplemental study that consisted of a control group and one single dose group (MRID 46214402). In the initial study, the high dose was reduced to a lower dose due to mortality and the supplemental study, with a single dose followed the same study protocol and methods.

In the initial study (MRID 46214401), Phorate (91.8% a.i., batch # AC 9429-41) was administered to pre-mated female Wistar (CrlGlxBrlHan:WI) rats/dose by gavage in corn oil at doses of 0, 0.03, 0.1 and 0.3 mg/kg bw/day from gestation day (GD) 6 through postnatal day (PND) 10 in a volume of 5 mL/kg body weight. Due to excessive mortality, the dosage of the 0.3 mg/kg/day group was reduced to 0.2 mg/kg/day on lactation day 3. Small litter size and increased pup mortality necessitated termination of this group before direct dosing of offspring commenced.

In the supplemental study (MRID 46214402), Phorate (91.8% a.i., batch # AC 9429-41) was administered to pre-mated female Wistar (CrlGlxBrlHan:WI) rats/dose by gavage in corn oil at doses of 0 and 0.2 mg/kg bw/day from gestation day (GD) 6 through postnatal day (PND) 10 in a volume of 5 mL/kg body weight. The test material was administered to offspring at the same doses from PNDs 11 through 21.

A Functional Observational Battery (FOB) was performed on 10 dams/dose on gestation days 7 and 14, and on 10 dams/dose on lactation days 7 and 14. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory, and neuropathology at study termination (day 60±2 of age). On postnatal day 22, the whole brain was collected from 10 pups/sex/dose group for micropathologic examination and morphometric analysis. Brain, erythrocyte, and serum cholinesterase activities were measured in offspring (10/dose group) on days 4 and 21 (3 hours after dosing) and in dams (10/dose group) on postnatal day 21. Pup physical development was

assessed by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No mortality or toxicity was seen when the high dose was reduced to 0.2 mg/kg/day. At the initial high dose (0.3 mg/kg/day) mortality was seen towards the end of gestation and during early lactation. Clinical signs of toxicity at 0.3 mg/kg/day dams included tremors, high stepping gait, poor general state, labored respiration, salivation after treatment and chromodacryorrhea . Body weight during gestation and lactation was only slightly affected in dams administered 0.3 mg/kg bw/day. In addition, body weight gain for the high-dose group was decreased during LDs 1-2. Body weight loss for this group was accompanied by decreased food consumption during early lactation. No treatment-related clinical signs of toxicity or effects on body weight and food consumption were observed in any treated groups, up to and including 0.2 mg/kg/day. No biologically significant inhibition of serum, erythrocyte (RBC) or brain ChE activities was found in dams on lactation day 21.

Based on the combined results of both studies, the maternal systemic LOAEL is 0.3 mg/kg bw/day based on death, clinical signs of toxicity, decreases in body weight gain during gestation and reduced food consumption during lactation. The maternal NOAEL is 0.2 mg/kg bw/day.

The maternal cholinesterase NOAEL is 0.2 mg/kg/day; the highest dose tested. A maternal cholinesterase LOAEL is not established.
No treatment-related effect on the mean number of pups delivered per dam was seen. However for females at 0.3 mg/kg bw/day, the number of live born pups was decreased and the number of stillborn pups was increased compared with the control group. Correspondingly, the live birth index was decreased for this treated group. Pup growth and viability were markedly decreased at 0.3 mg/kg bw/day during LDs 1-4 probably as a consequence of maternal neglect. Although the high dose was reduced at the end of gestation/early lactation, maternal mortality and small litter size due to increased pup mortality necessitated termination of this group before direct dosing of offspring commenced. Offspring survival in the other treated groups was similar to the controls and no clinical signs of toxicity were observed.

In the 0.3 mg/kg bw/day group, male and female offspring body weights were lower than those of the controls on PNDs 1 and 4, and body weight gains were decreased during this interval. A trend for decreased weight gain by males in the 0.1 and 0.2 mg/kg bw/day groups is suggested during late lactation. However, the magnitude of the differences in the mean values between the treated and control groups was less than the standard deviation and was about the mean for the treated group at each interval that attained statistical significance. In addition, absolute body weight was not affected at any time during lactation, apparent differences in weight gain were found only for overnight intervals, and statistical analysis may not have been appropriate. Therefore, decreased body weight gain by male pups at 0.1 and 0.2 mg/kg bw/day is not considered treatment-related or biologically significant. During the post-weaning interval, body weight and body weight data were considered separately for each subset. Although statistical significance was occasionally found for body weight or body weight gain during the post-weaning interval, no dose- or treatment-related trends were observed. The mean day of sexual maturation and body weight at attainment were not affected by treatment.

No treatment-related effects were seen on clinical signs, FOB parameters, developmental landmarks,, brain weights, brain morphology or neuropathology.

No conclusions can be made with regard to the effect of phorate on offspring learning and memory due to conflicting results between the two studies. When the same testing methods used, learning was apparent in the main study but not in the supplemental study. Consequently, no confidence can be placed in the results obtained in the assessment of this parameter.

A treatment-related increase in the motor activity was seen in both sexes of pups on PND 21 at the at 0.1 mg/kg/day. The increase, over controls, were 38% in males and 21% in females. An increase is also noted at the low dose (0.03 m/k/d) in males (9%) and females (10%); however, these increases were determined to be not toxicologically significant due to the lesser magnitude of the effect.

A clear treatment-related decreases in mean peak amplitude response was seen in the males on PND 60 at 0.1 mg/kg/day. This decrease is supported by a smaller magnitude of decrease in males on PND 24 and by habituation data. The effect is consistent across blocks. Data from the follow-up study at 0.2 mg/kg/day support the results seen at 0.1 mg/kg/.day. No treatment-related inhibition of serum, RBC, or brain ChE activity was found in either sex on PND 4. At 0.1 mg/kg/day significant inhibition was seen in plasma (15%), RBC (16%) and brain (21%) in males on Day 21; only plasma (19%) was inhibited in females on that day. At 0.2 mg/kg/day, significant inhibitions were seen in all three compartments.

The offspring systemic and cholinesterase inhibition LOAEL is 0.1 mg/kg/day based on increases in motor activity in both sexes on PND 21, decrease in auditory startle reflex in males on PND 60 and plasma, RBC and brain cholinesterase inhibition in males on Day 21. The offspring NOAEL is 0.03 mg/kg/day.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessment of learning and memory in the offspring and the pending review of the of positive control data.

DATA EVALUATION RECORD

PHORATE

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRIDS 46214401 (main study), 46214402 (supplemental study)

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 No. 35-2004

Robert H. Ross

Primary Reviewer:
Virginia A. Dobozy, V.M.D., M.P.H.
Signature:
Date: JUN 14 2004

Secondary Reviewers:
Carol S. Wood, Ph.D., D.A.B.T.
Signature: Carol S Wood
Date: JUN 14 2004

Robert H. Ross, M.S. Group Leader
Signature: Robert H. Ross
Date: JUN 14 2004

Quality Assurance:
Lee Ann Wilson, M.A.
Signature: L.A. Wilson
Date: JUN 14 2004

Disclaimer

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

Oak Ridge National Laboratory is managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: William Greear, M.P.H., D.A.B.T. **Signature:** William Greear
Registration Action Branch 1, Health Effects Division (7509C) **Date** 9/19/2005
EPA Work Assignment Manager: PV Shah, Ph.D.. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 10/5/05

TXR#: 0052433

DATA EVALUATION RECORD

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

PC CODE: 057201 **DP BARCODE:** D300057
SUBMISSION NO.:

TEST MATERIAL (PURITY): Technical Grade Phorate (91.8%)

SYNONYMS: BAS 225 I; O,O-diethyl S-ethylthiomethyl phosphorodithioate

CITATION: Kaufmann, W., S. Schneider, K. Deckardt, *et al* (2004) BAS 225 I (phorate) - Developmental Neurotoxicity Study in Wistar Rats Oral Administration to the Dams and Pups (Gavage). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Project I.D. 66R0091/02039; February 23, 2004. MRID 46214401. Unpublished

Kaufmann, W., S. Schneider, K. Deckardt, *et al* (2004) BAS 225 I (phorate) - Developmental Neurotoxicity Study in Wistar Rats Oral Administration to the Dams and Pups (Gavage) (Supplemental Report - Additional Dose Level). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Project I.D. 66R0091/02052; February 23, 2004. MRID 46214402. Unpublished

SPONSOR: BASF Corporation, Agricultural Products Division, RTP, NC.

EXECUTIVE SUMMARY:

NOTE: This Data Evaluation Record contains the review of two developmental neurotoxicity studies. The initial study that tested three dose level (MRID 46214401) and a supplemental study that consisted of a control group and one single dose group (MRID 46214402). In the initial study, the high dose was reduced to a lower dose due to mortality and the supplemental study, with a single dose followed the same study protocol and methods.

In the initial study (MRID 46214401), Phorate (91.8% a.i., batch # AC 9429-41) was administered to pre-mated female Wistar (CrlGlxBrlHan:WI) rats/dose by gavage in corn oil at doses of 0, 0.03, 0.1 and 0.3 mg/kg bw/day from gestation day (GD) 6 through postnatal day (PND) 10 in a volume of 5 mL/kg body weight. Due to excessive mortality, the dosage of the 0.3 mg/kg/day group was reduced to 0.2 mg/kg/day on lactation day 3. Small litter size and increased pup mortality necessitated termination of this group before direct dosing of offspring commenced.

In the supplemental study (MRID 46214402), Phorate (91.8% a.i., batch # AC 9429-41) was administered to pre-mated female Wistar (CrlGlxBrlHan:WI) rats/dose by gavage in corn oil at doses of 0 and 0.2 mg/kg bw/day from gestation day (GD) 6 through postnatal day (PND) 10 in a volume of 5 mL/kg body weight. The test material was administered to offspring at the same doses from PNDs 11 through 21.

A Functional Observational Battery (FOB) was performed on 10 dams/dose on gestation days 7 and 14, and on 10 dams/dose on lactation days 7 and 14. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory, and neuropathology at study termination (day 60±2 of age). On postnatal day 22, the whole brain was collected from 10 pups/sex/dose group for micropathologic examination and morphometric analysis. Brain, erythrocyte, and serum cholinesterase activities were measured in offspring (10/dose group) on days 4 and 21 (3 hours after dosing) and in dams (10/dose group) on postnatal day 21. Pup physical development was assessed by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No mortality or toxicity was seen when the high dose was reduced to 0.2 mg/kg/day. At the initial high dose (0.3 mg/kg/day) mortality was seen towards the end of gestation and during early lactation. Clinical signs of toxicity at 0.3 mg/kg/day dams included tremors, high stepping gait, poor general state, labored respiration, salivation after treatment and chromodacryorrhea . Body weight during gestation and lactation was only slightly affected in dams administered 0.3 mg/kg bw/day. In addition, body weight gain for the high-dose group was decreased during LDs 1-2. Body weight loss for this group was accompanied by decreased food consumption during early lactation. No treatment-related clinical signs of toxicity or effects on body weight and food consumption were observed in any treated groups, up to and including 0.2 mg/kg/day. No biologically significant inhibition of serum, erythrocyte (RBC) or brain ChE activities was found in dams on lactation day 21.

Based on the combined results of both studies, the maternal systemic LOAEL is 0.3 mg/kg bw/day based on death, clinical signs of toxicity, decreases in body weight gain during gestation and reduced food consumption during lactation. The maternal NOAEL is 0.2 mg/kg bw/day.

The maternal cholinesterase NOAEL is 0.2 mg/kg/day; the highest dose tested. A maternal cholinesterase LOAEL is not established.

No treatment-related effect on the mean number of pups delivered per dam was seen. However for females at 0.3 mg/kg bw/day, the number of live born pups was decreased and the number of stillborn pups was increased compared with the control group. Correspondingly, the live birth index was decreased for this treated group. Pup growth and viability were markedly decreased at 0.3 mg/kg bw/day during LDs 1-4 probably as a consequence of maternal neglect. Although the high dose was reduced at the end of gestation/early lactation, maternal mortality and small litter size due to increased pup mortality necessitated termination of this group before direct dosing of offspring commenced. Offspring survival in the other treated groups was similar to the controls and no clinical signs of toxicity were observed.

In the 0.3 mg/kg bw/day group, male and female offspring body weights were lower than those of the controls on PNDs 1 and 4, and body weight gains were decreased during this interval. A trend for decreased weight gain by males in the 0.1 and 0.2 mg/kg bw/day groups is suggested during late lactation. However, the magnitude of the differences in the mean values between the treated and control groups was less than the standard deviation and was about the mean for the treated group at each interval that attained statistical significance. In addition, absolute body weight was not affected at any time during lactation, apparent differences in weight gain were found only for overnight intervals, and statistical analysis may not have been appropriate. Therefore, decreased body weight gain by male pups at 0.1 and 0.2 mg/kg bw/day is not considered treatment-related or biologically significant. During the post-weaning interval, body weight and body weight data were considered separately for each subset. Although statistical significance was occasionally found for body weight or body weight gain during the post-weaning interval, no dose- or treatment-related trends were observed. The mean day of sexual maturation and body weight at attainment were not affected by treatment.

No treatment-related effects were seen on clinical signs, FOB parameters, developmental landmarks,, brain weights, brain morphology or neuropathology.

No conclusions can be made with regard to the effect of phorate on offspring learning and memory due to conflicting results between the two studies. When the same testing methods used, learning was apparent in the main study but not in the supplemental study. Consequently, no confidence can be placed in the results obtained in the assessment of this parameter.

A treatment-related increase in the motor activity was seen in both sexes of pups on PND 21 at the at 0.1 mg/kg/day. The increase, over controls, were 38% in males and 21% in females. An increase is also noted at the low dose (0.03 m/k/d) in males (9%) and females (10%); however, these increases were determined to be not toxicologically significant due to the lesser magnitude of the effect.

A clear treatment-related decreases in mean peak amplitude response was seen in the males on PND 60 at 0.1 mg/kg/day. This decrease is supported by a smaller magnitude of decrease in males on PND 24 and by habituation data. The effect is consistent across blocks. Data from the follow-up study at 0.2 mg/kg/day support the results seen at 0.1 mg/kg/.day. No treatment-related inhibition of serum, RBC, or brain ChE activity was found in either sex on PND 4. At 0.1 mg/kg/day significant inhibition was seen in plasma (15%), RBC (16%) and brain (21%) in

males on Day 21; only plasma (19%) was inhibited in females on that day. At 0.2 mg/kg/day, significant inhibitions were seen in all three compartments.

The offspring systemic and cholinesterase inhibition LOAEL is 0.1 mg/kg/day based on increases in motor activity in both sexes on PND 21, decrease in auditory startle reflex in males on PND 60 and plasma, RBC and brain cholinesterase inhibition in males on Day 21. The offspring NOAEL is 0.03 mg/kg/day.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessment of learning and memory in the offspring and the pending review of the of positive control data.

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

I. MATERIALS AND METHODS (MRIDs 46214401 and 46214402):

A. MATERIALS:

1. Test material: Technical grade Phorate

Description:	Liquid/clear to slightly turbid
Batch #:	AC 9429-41
Purity:	91.8 % a.i.
Compound Stability:	expiry date December 2003; proven by reanalysis
CAS # of TGAI:	298-02-2

2. Vehicle: corn oil

3. Test animals (P):

Species:	Rat
Strain:	Wistar (CrlGlxBrlHan:WI)
Age at study initiation:	Time-mated females: 10-12 wks
Wt. at study initiation:	149.8-193.4 g (on post-coital day 0)
Source:	Charles River Laboratories, Germany
Housing:	Individually in stainless steel cages, except from gestation day 18 to lactation day 21 when dams with litters were in Makrolon type M III cages
Diet:	ground or pelleted Kliba maintenance diet rat/mouse/hamster (Provimi Kliba SA, Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 20-24°C **Humidity:** 30-70% **Air changes:** Not provided **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	Six days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: January 20, 2003; End: April 23, 2003 (MRID 46214401)
   Start: April 7, 2003; End: July 1, 2003 (MRID 46214402)

2. **Study schedule:** Time-mated female Wistar rats (45/dose group) were administered the test material by gavage from gestation day (GD) 6 through postnatal day (PND) 10. On postnatal day 4, litters were standardized to 8 pups, sexes were represented as equally as possible. Pups were weaned from the dam on PND 21; dams were sacrificed after weaning. The test material was administered by gavage to pups from PND 11 through PND 21. Pups remained on study up to PND 62.

3. **Mating procedure:** Time mating of females was carried out at Charles River Laboratories, Germany. The day that a vaginal plug or sperm in a vaginal smear was detected was designated gestation day (GD) 0. Females presumed to be pregnant were delivered to the testing laboratory on GD 0.

4. **Animal assignment:** The mated females were randomly assigned to treatment groups upon arrival at the testing laboratory, as shown in Table 1. The method used for assigning animals

was not stated. Due to excessive toxicity, the high dose was reduced to 0.2 mg/kg bw/day during late gestation or early lactation; however this group was terminated before initiation of direct dosing of pups. On GDs 7 and 14 and lactation days (LDs) 7 and 14, 10 females/group were examined outside the cage using a functional observation battery (FOB) of tests.

Eight subsets of 10 pups/sex/group were assigned for detailed clinical observations (FOB), measurements of motor activity, auditory startle, learning and memory, cholinesterase activity and neuropathology as noted in Table 1 for MRID 46214401 (definitive study). The same study design was used for MRID 46214402 (supplementary study), except there were only two doses, control and 0.2 mg/kg bw/day.

TABLE 1. Study design

Experimental parameter	Dose (mg/kg bw/day)			
	0	0.03	0.1	0.3/0.2
Maternal animals				
	No. of maternal animals assigned			
No. of maternal animals assigned	45	45	45	45
FOB (GDs 7 and 14, LDs 7 and 14)	10	10	10	10
Offspring				
	No. of offspring assigned			
Subset I - Immersion fixation, brain preservation (PND 11)	10/sex	10/sex	10/sex	10/sex
Subset II - Perfusion fixation, brain weight and neuropathology (PND 22)	10/sex	10/sex	10/sex	10/sex
Subset III - Auditory startle test (LDs 24, 60), perfusion fixation, brain weight, neuropathology (PND 62)	10/sex	10/sex	10/sex	10/sex
Subset IV - FOB (LDs 4, 11, 21, 35, 45, 60), motor activity (PNDs 13, 17, 21, 60)	10/sex	10/sex	10/sex	10/sex
Subset V - Learning and memory test (water maze test) (PNDs 23 and 30)	10/sex	10/sex	10/sex	10/sex
Subset VI - Learning and memory test (water maze test) (PNDs 60 and 67)	10/sex	10/sex	10/sex	10/sex
Subset VII - Cholinesterase measurements (PND 4)	10/sex	10/sex	10/sex	10/sex
Subset VIII - Cholinesterase measurements (PND 21)	10/sex	10/sex	10/sex	10/sex

5. **Dose selection rationale:** No dose selection rationale was provided for the initial study that tested three dose levels. The test dose (0.2 mg/kg/day) for the supplementary study was based on maternal and offspring toxicity at the high dose (0.3 mg/kg bw/day) in the initial study.

6. **Dosage administration:** Phorate was administered to maternal animals by gavage on GD 6 through lactation day 10, in a volume of 5 mL/kg of body weight. Dosing was based on the most recent body weight determination. The test material was administered to pups by

gavage at the same doses as dams from PNDs 11 through 21. Dosage of the 0.3 mg/kg bw/day group was reduced to 0.2 mg/kg bw/day on lactation day 3.

7. **Dosage preparation and analysis:** Formulations were prepared on the day of administration by mixing appropriate amounts of test substance with corn oil. Prior to the start of the study, stability of the test substance in corn oil was evaluated for a period of at least 12 days at room temperature. Homogeneity was not evaluated. During the study, samples of the oily test substance solutions were analyzed three times for concentration.

Results:

Homogeneity analysis: Homogeneity was not determined; the preparations were noted to be true solutions.

Stability analysis: The test material was stable in corn oil at room temperature for 12 days (102% and 102.8% of the initial value in MRIDs 46214401 and 46214402, respectively).

Concentration analysis: In MRID 46214401, the concentration ranges for the 0.03, 0.1 and 0.3/0.2 mg/kg bw/day doses were 92.4-109.1%, 90.9-95.5%, and 92.4-93.2% of nominal, respectively. In MRID 46214402, the concentration of the 0.2 mg/kg bw/day dose was 90-99% of nominal.

The analytical data indicated that the concentration and stability of phorate in the corn oil preparations were adequate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Twice daily checks for mortality or moribundity and daily cage-side observations were conducted on maternal animals. Gross observations of the dams were conducted daily, prior to treatment. Signs of toxicity were recorded as they were observed, including the time of onset, degree, and duration.

Ten dams per group were observed outside the home cage at least twice during the gestation period (days 7 and 14) and twice during the lactation period (days 7 and 14). The standard arena was 50 × 37.5 cm with a lateral border of 25 cm. No other experimental details were given. The following functional observations were recorded.

	Functional observations–Maternal animals
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 exophthalmos; 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; 6) Respiration; 7) Activity/arousal level.
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.

Individual maternal body weight was recorded upon arrival at the testing facility (GD 0) and on GDs 6-20. Females with litters were weighed on the day of parturition and on LDs 10, 14 and 21. Food consumption measurements were recorded on GDs 0, 6, 13 and 20 and on LDs 1, 7, 14 and 21.

Blood samples were collected from the retro orbital venous flexus under isoflurane anesthesia from 10 dams per group on LD 21 for serum and red blood cell cholinesterase measurements. Brain cholinesterase was measured on the same animals on LD 21.

b. Offspring:

1) **Litter observations:** The day of completion of parturition was designated as PND 0. Live pups were counted, sexed and weighed individually for each litter on PNDs 1, 4 (before standardization) and 11-21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration.

   On PND 4, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible), with the exception of the 0.3/0.2-mg/kg bw/day group; excess pups were killed and discarded. Litters with fewer than 8 pups were removed from the study.

2) **Developmental landmarks:** Beginning on postnatal day 40, male offspring were examined daily for preputial separation. Beginning on postnatal day 27, female offspring were examined daily for vaginal patency. The age of onset and the offspring body weight at that time were recorded.

3) **Postweaning observations:** After weaning on PND 21, offspring were examined twice daily for mortality, and cage-side observations were conducted once daily. Individual offspring body weight data were recorded weekly.

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

I) **Functional observational battery (FOB) (subset IV):** On PNDs 4, 11, 21, 35, 45, and 60, ten offspring/sex/group (one male or one female from each litter) were examined outside the home cage in an FOB assessment. The same parameters assessed in the maternal FOB were examined for offspring, as appropriate for the developmental stage being observed. The standard arena was 50 x 37.5 cm with a lateral border of 25 cm. No other experimental details were given.

ii) **Motor activity testing (subset IV):** Motor activity was evaluated in 10 pups/sex/dose on days 13, 17, 21 and 60 using the Tru Scan Photobeam Linc. The activity was measured in 10 enclosures in randomized order. Each enclosure was equipped with two sensor rings each with 16 light beams per cage side. The distance covered and the number of rearings were recorded over 12 intervals, each lasting 5 minutes. No food or water was provided and the room was darkened during the measurements.

iii) **Auditory startle reflex habituation (subset III):** Auditory startle reflex habituation testing was performed on 10 offspring/sex/dose on postnatal days 24 and 60, using the SR-LAB; STARTLE RESPONSE SYSTEM. The animals were allowed a 5 minute acclimation period in the response chamber with a 70dBA background noise. The startle response was recorded in 50 trials with a startle stimulus sound level of 120 dBA with a 5 second interval between the trials. Response was recorded for 50 milliseconds. Maximum amplitude and latency to peak response were analyzed in 5 blocks of 10 trials each.

iv) **Learning and memory testing (subsets V, VI):** Learning and memory testing was performed in 10 offspring/sex/dose. Water maze testing was performed on PNDs 23 and 30 (subset V) and on PNDs 60 and 67 (subset VI). The testing consisted of three parts and was performed in two weeks, beginning with learning ability (learning 1) in the first week, followed by memory and relearning ability (learning 2) in the second week. Both studies followed the same testing procedures.

The learning 1 test consisted of 6 trials at intervals of 1 hour for each animal. At each trial, the animals were required to find an escape ladder on the right side of an M shaped water maze pool. The maximum duration of swimming was 6 minutes per trial. A positive score (+) was given if the animal found the escape immediately. If the animal went in the wrong direction, it was given a negative (-) score but left in the water until it found the ladder or the 6 minutes expired. The time needed to find the ladder was also recorded.

For the memory test, the same animals had to find the ladder on the right side of the pool after one week; the time needed to find the ladder was recorded.

The learning 2 test started 1 hour after completion of the memory test. The same procedures were followed as in learning test 1, except the ladder was placed on the left side of the maze. The initial trials in the learning 1 and 2 tests were not included in the analyses since they served as an acclimation to the test.

5) **Cholinesterase determination:** Cholinesterase (serum, red blood cell and brain) activity was determined on PNDs 4 (using culled pups) and 21 (3 hours after dosing, 1 pup/sex/litter). Blood samples were collected after decapitation from the vena cava cranialis following isoflurane anesthesia. The same animals were used for blood and brain analyses. The blood and brain samples were analyzed using a spectrophotometric procedure, based on modified Ellman's methods that were adapted to a Cobas Fara analyzer. Red blood cell samples were measured using DTNA as chromogen. Blood and brain samples were kept on ice during collection and processed as soon as possible. After all the brains were collected, the samples were deep frozen and stored at -80^{0}C until analysis. Hematocrit and protein content of the brain were determined in order to calculate the cholinesterase activity of red blood cells per liter and specific cholinesterase activity of the brain, respectively.

2. **Postmortem observations**:

a. **Maternal animals:** Dams not used for cholinesterase activity measurements were sacrificed by cervical dislocation and discarded without examination on PND 21 after the pups were weaned. Animals without a litter were discarded after the uterus had been stained for evidence of early resorptions. To determine the number of implantation sites, the uterus was stained with 10% ammonium sulfide solution.

b. **Offspring:** All pups that died and had been placed in a subset were examined externally, eviscerated and their organs assessed macroscopically and fixed in 4% formaldehyde. All pups sacrificed on schedule (pups sacrificed on PND 21 and subset IV, V and VI) were killed by cervical dislocation and discarded without examination.

On PND 11, 10 animals/sex/group were subjected to deep anesthesia and sacrificed by exsanguination. The skull was separated from the body and stored in neutrally buffered 4% formaldehyde.

At postnatal days 22, ten pups/sex/group were first weighed and then deeply anesthetized and sacrificed by perfusion fixation. SOERENSEN phosphate buffer was used as a rinsing solution and neutrally buffered 4% formaldehyde as a fixative. The animals were necropsied and the visible organs assessed by gross pathology. The cranial vault and spinal cord were opened and the skin removed from both hind extremities. The perfused animals were stored in a neutrally buffered 4% formaldehyde solution for at least 48 hours. Brain (with olfactory bulb) weight was determined after removal of the organ. The length and maximum width of the cerebrum and cerebellum were measured. Brains from all dose groups of both studies were embedded in paraffin. In MRID 46214402, tissues from the brain, spinal cord, eyes, pituitary gland, olfactory epithelium,

gasserian ganglia, and gastrocnemius muscle were sectioned, stained with hemotoxylin and eosin and examined by light microscopy. In MRID 46214401, only sections of the frontal lobe (males and females) and cerebellum (females) were stained and examined.

Morphometric measurements of major brain areas were done in MRID 46214401 only as indicated for those brain regions where a significant deviation was found at 0.2 mg/kg bw/day of the supplementary study. The following brain morphometric measurements were performed in MRID 46214402:

Neocortex thickness (width of the total cortical mantle measured vertically to a tangent over a region of the frontal and parietal cortices determined beforehand) (MRID 46214401: males, left frontal and parietal cortex; females, right frontal cortex)

Caudate nucleus/putamen (largest lateral extension of the left and the right part was determined)

Corpus callosum (width measured as the middle line of the cross section)

Hippocampus (largest dorsoventral extension)

Cerebellum (width of a select folium measured in the middle of a line which runs vertically to a tangent from the tip to the base of the folium) (MRID 46214401: females, folium pyramis)

On PND 60±2, ten pups/sex/group were first weighed and then deeply anesthetized and sacrificed by perfusion fixation. SOERENSEN phosphate buffer was used as a rinsing solution and neutrally buffered 4% formaldehyde as a fixative. The animals were necropsied and the visible organs assessed by gross pathology. The cranial vault and spinal cord were opened and the skin removed from both hind extremities. The perfused animals were stored in a neutrally buffered 4% formaldehyde solution for at least 48 hours. Brain (with olfactory bulb) weight was determined after removal of the organ. The length and maximum width of the cerebrum and cerebellum were measured. The brain, spinal cord, eyes, pituitary gland, nasal cavity, gasserian ganglia, gastrocnemius muscle, dorsal root fibers and ganglia, ventral root fibers, and peripheral nerves (sciatic and tibial) were collected and preserved in fixative.

In MRID 46214402, the following central and peripheral nervous system tissues were dissected and embedded in paraffin (CNS tissues) or plastic (PNS tissues): olfactory bulb, frontal lobe, parietal lobe with diencephalon, midbrain with occipital and temporal lobe, pons, cerebellum (2 planes), medulla oblongata, spinal cord, eyes, pituitary gland, olfactory epithelium, gasserian ganglia, gastrocnemius muscle, dorsal root ganglion, dorsal root fiber, ventral root fiber, proximal sciatic nerve, and proximal and distal tibial nerve. Paraffin-embedded tissues were sectioned, stained with hemotoxylin and eosin, and examined by light microscopy. Semi-thin sections of plastic-embedded tissues were

examined by light microscopy. In MRID 46214401, brains were embedded but not sectioned.

The following brain morphometric measurements were performed in MRID 46214402:

Neocortex thickness (width of the total cortical mantle measured vertically to a tangent over a region of the frontal and parietal cortices determined beforehand)

Caudate nucleus/putamen (largest lateral extension of the left and the right part was determined)

Corpus callosum (width measured as the middle line of the cross section)

Hippocampus (largest dorsoventral extension)

Cerebellum (width of a select folium measured in the middle of a line which runs vertically to a tangent from the tip to the base of the folium)

D. DATA ANALYSIS:

1. **Statistical analyses:** The following parameters were analyzed using the Dunnett's test (two-sided) for the hypothesis of equal means: food consumption (females), body weight and body weight gain (females and pups), duration of gestation and number of pups delivered per litter.

   The following were analyzed using the Fisher's Exact test for hypothesis of equal proportions: female fertility index, gestation index, females with live born pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, and water maze evaluation.

   The Wilcoxon test (one-sided) for the hypothesis of equal medians was also used for water maze evaluation.

   Motor activity and startle response were analyzed using Kruskal-Wallis test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of the dose groups with the control was performed using Mann-Whitney U-test (two-sided) for the hypothesis of equal medians.

   Cholinesterase and brain weight (absolute and relative) data were analyzed using Kruskal-Wallis test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of the dose groups with the control was performed using Wilcoxon test (two-sided) for the hypothesis of equal medians.

   Morphometric parameters were analyzed using the Wilcoxon-test (one-sided) with Bonferoni-Holm-Adjustment for the hypothesis of equal medians.

2. **Indices:**

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

Female fertility index (%) = number of pregnant females* x 100
number of females mated**

* defined as number of females that gave birth to a litter or with pups/fetuses *in utero*
** defined as number of females with vaginal sperm or that gave birth to a litter or with fetuses *in utero*

Gestation index (%) = number of females with live pups on the day of birth x 100
number of females pregnant*

* defined as the number of females that gave birth to a litter or with fetuses *in utero*

Live birth index (%) = number of live born pups at birth x 100
number of pups born

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

Viability index (%) = number of live pups on day 4* after birth x 100
number of live pups on day of birth

* before standardization of litters

Lactation index (%) = number of live pups on day 21 after birth x100
number of live pups on day 4* after birth

* after standardization of litters

Sex ratio = number of live male or female pups on day 0/21 x 100
number of live male and female pups on day 0/21

3. **Positive and historical control data:** Positive control data are under review.

II. RESULTS

NOTE: **The results of the initial study (MRID 46214401) with the three dose levels are discussed below. In this study, due to severe maternal mortality, the initial high dose (0.3 mg/kg/day) was reduced to 0.2 mg/kg/day on LD 6**

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** A total of six dams at the high dose (0.3 mg/kg/day) were found dead on GD 21 and 22 and LD 1. Slight tremor was the only clinical observation in one of these animals prior to death. Apparently, no postmortem examinations were conducted; no data were submitted. Due to these deaths, the high dose was reduced to 0.2 mg/kg/day on LD 6. No mortality occurred at this dose.

   Among survivors, tremors were noted in two dams at 0.3 mg/kg bw/day during gestation (GD 21). Salivation after treatment was observed in two dams at 0.3 mg/kg bw/day during gestation (GDs 17 and 18). FOB observations considered treatment-related included slight tremors in two high dose females on GD 14. Clinical signs noted during LDs 0-4 in five of the high dose group (0.3 mg/kg/day) included tremors, high stepping gait, poor general state and labored respiration. Two animals at this dose also had salivation after treatment and chromodacryorrhea; one animal had a red crusty formation on the nose.

   During days 0-4 of lactation, 9 females in the high dose group had increased numbers of pup deaths and 11 had total litter loss. The high dose was discontinued before the initiation of direct pup dosing. No clinical signs of toxicity was seen at 0.2 mg/kg/day. These results are summarized in Table 2.

TABLE 2. Maternal mortality and clinical/functional observations (number of animals affected)[a]					
Observation	Dose (mg/kg bw/day)				
	0	0.03	0.1	0.2	0.3
Gestation					
Mortality	0	0	0	0	4
Tremors	0	0	0	0	3
Salivation after treatment	0	0	0	0	2
Lactation					
Mortality	0	0	0	0	2
Tremors	0	0	0	0	5
High stepping gait	0	0	0	0	5
Chromodacryorrhea	0	0	0	0	2
General poor state of health	0	0	0	0	5
Nose, red crusty formation	0	0	0	0	1
Salivation after treatment	0	0	0	0	2
Labored respiration	0	0	0	0	5
Pups not properly nursed	0	0	0	0	9
Total litter loss	0	0	0	0	11

[a] Data obtained from pages 100-103, MRID 46214401.

2. **Body weight and food consumption**: Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 3. Mean body weight in treated groups was comparable to controls during gestation but was slightly decreased in females at 0.3/0.2 mg/kg bw/day on LDs 0 (95% of control value) and 2 (94% of control value). Mean body weight gain was slightly decreased in females at 0.3/0.2 mg/kg bw/day on GDs 7-8, 9-10 and 19-20. Mean weight gain during the treatment period of gestation (GDs 6-20) was decreased (90% of control value) in the high dose group. Mean weight gain during lactation was decreased at 0.3 mg/kg bw/day for days 1-2 (-3.8 g vs. 1.0 g in control).

   Food consumption in treated animals was comparable to controls during gestation but was significantly decreased (84% of control value) in the high dose group on days 1-7 of lactation.

TABLE 3. Selected mean (±SD) maternal body weight and food consumption[a]				
Observations/study interval	Dose (mg/kg bw/day)			
	0	0.03	0.1	0.3/0.2
Gestation (n= 31-40)				
Body wt. Gestation day 0 (g)	169.3±9.8	172.5±11.6	169.7±10.2	171.2±9.7
Body wt. Gestation day 7 (g)	198.6±11.0	201.4±12.4	197.8±12.7	198.5±10.6
Body wt. Gestation day 15 (g)	232.8±13.1	235.3±16.2	231.2±16.7	230.2±12.4
Body wt. Gestation day 20(g)	279.3±16.1	282.5±21.0	277.6±22.8	270.7±17.3
Wt. gain gestation days 6-20 (g)	82.8±10.8	83.0±10.2	82.3±12.5	74.4*±13.3 (90)
Food consumption gestation days 0-6 (g/animal/day)	16.7±1.0	16.6±1.2	16.3±1.4	16.5±1.2
Food consumption gestation days 6-20 (g/animal/day	17.8±0.8	17.8±1.1	17.3±0.8	17.6±0.8
Lactation (n=36-38)[b]				
Body wt. lactation day 0 (g)	223.0±14.8	224.0±18.2	217.2±14.4	211.8**±16.0 (95)
Body wt. lactation day 2 (g)	217.8±15.8	220.9±18.4	214.4±15.7	205.7**±12.9 (94)
Body wt. lactation day 10 (g)	246.0±16.3	249.3±15.9	243.4±17.7	241.1±3.6
Body wt. lactation day 21 (g)	245.7±14.1	247.1±15.0	242.4±17.2	NA
Wt gain lactation days 0-1 (g)	-6.7±9.2	-3.3±8.0	-5.7±8.7	-6.1±8.5
Wt gain lactation days 1-2 (g)	1.0±4.7	0.6±5.5	3.3±5.8	-3.8*±8.4
Wt gain lactation days 0-21(g)	23.3±9.1	22.8±10.1	24.5±13.0	NA
Food consumption lactation days 1-7 (g/animal/day)	28.8±3.2	29.4±3.4	29.5±3.4	24.3**±4.6 (84)

[a]Data obtained from pages 104-113, MRID 46214401
[b]Number of females in high dose decreased from 36 on LD 0 to 5 on LD 10.
NA= Not applicable, dose discontinued
* Statistically significantly different from control, p≤0.05
** Statistically significantly different from control, p≤ 0.01.
Number in parentheses is % of control value, calculated by reviewer.

3. **Reproductive performance**: The following number of females in the control, 0.03, 0.1 and 0.3/0.2 mg/kg bw/day groups did not deliver pups: 7, 5, 4 and 5, respectively. The fertility index was 84% (control group), 89% (low and high dose group) and 91% (mid dose group). The mean duration of gestation was comparable between control and treated groups. The gestation index was 100%, 98%, 100% and 88% in the control, 0.03, 0.1 and 0.3/0.2 mg/kg bw/day groups, respectively. Results for the maternal animals are summarized in Table 4.

TABLE 4. Reproductive performance[a]				
Observation	Dose (mg/kg bw/day)			
	0	0.03	0.1	0.3/0.2
Number mated	45	45	45	45
Number pregnant	38	40	41	40
Fertility index (%)	84	89	91	89
Intercurrent deaths	0	0	0	6
Gestation index (%)	100	98	100	88
Mean (±SD) gestation duration (days)	21.6±0.5	21.9±0.7	21.6±0.6	21.8±0.6
Incidence of dystocia	0	0	0	0

[a] Data obtained from page114, MRID 46214401.

4. **Cholinesterase activity**: There was no evidence of treatment-related inhibition of cholinesterase activity in dams on LD 21. The data are included with offspring data in Table 15.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in the Table 5. The mean number of delivered pups per dam was not affected by treatment. The numbers of live born and stillborn pups were significantly decreased and increased, respectively, in females at 0.3/0.2 mg/kg bw/day. The live birth index was decreased at 0.3/0.2 mg/kg bw/day. Pup viability was markedly decreased at 0.3/0.2 mg/kg bw/day during LDs 1-4. The total number of dead pups during this period was 1, 2, 3 and 66 for the control, 0.03, 0.1 and 0.3/0.2 mg/kg bw/day groups, respectively. Another 18 high dose pups were sacrificed due to dam mortality. Approximately 41% of the high dose pups died secondary to maternal toxicity. As a consequence, the high dose group was discontinued before direct dosing of the pups was begun.

   The sex ratio of live pups on the day of birth was not affected by treatment. There were no treatment-related clinical signs of toxicity.

TABLE 5. Litter size and viability [a]				
Observation	Dose (mg/kg bw/day)			
	0	0.03	0.1	0.3/0.2
Total number born	330	346	359	298
Pups/dam delivered	8.7±1.4	8.6±1.9	8.8±2.1	8.3±1.8
Number of litters	38	40	41	36
Number with live born litters	38	39	41	35
Number with stillborn pups	4	4	3	13**
Number born live	326	337	353	259**
Number born dead	4	9	6	39**
Sex Ratio Day 0 (% ♂)	49.4	48.1	42.8	46.3
# Deaths Days 1-4 (%)	1 (0.3)	2 (0.6)	3 (0.8)	66 (25)
# Deaths Days 5-7 (%)	0	0	0	NA
# Deaths Days 8-14	3 (0.9)	2 (0.6)	2 (0.6)	NA
# Deaths Days 15-21	1 (0.3)	4 (1.2)	7 (2.0)	NA
Mean litter size:				
Day 0	8.6±1.3	8.4±2.4	8.6±2.2	7.2±2.7
Day 4 [b]	8.6±1.3	8.4±2.3	8.4±2.5	4.9±4.1
Day 4 [c]	6.5±3.1	6.2±3.4	5.8±3.6	4.2±3.4
Day 11	6.4±3.1	6.2±3.4	5.8±3.6	1.0±2.6
Day 17	5.9±2.9	5.5±3.1	5.1±3.2	NA
Day 21	5.8±2.8	5.5±3.1	5.0±3.2	NA
Live birth index [d]	99	97	98	87

[a] Data obtained from pages 115-117 in the study report, MRID 46214401.
[b] Before standardization (culling).
[c] After standardization (culling).
[d] Calculated by the reviewer.
NA= not applicable, dose discontinued
** Statistically different from control, $p<0.01$

2. **Body weight:** Male and female offspring body weights were slightly lower at 0.3/0.2 mg/kg bw/day on PND 1 and slightly lower on PND 4 (pre- and post-culling). Body weight gain was slightly decreased (86% of control value) in high-dose males during PNDs 1-4. There was also a slight decrease (89% and 92% of control values) in body weight gain of males and females, respectively, at 0.1 mg/kg bw/day during PNDs 20-21; however, there was no effect for the entire lactation period. Selected mean preweaning pup body weight data are presented in Table 6.

TABLE 6. Selected mean (±SD) pre-weaning pup body weights and body weight gain [a]

PND	Dose (mg/kg bw/day)							
	0	0.03	0.1	0.3/0.2	0	0.03	0.1	0.3/0.2
	Males				Females			
Body Weight (g)								
1	6.5±0.5	6.6±0.6	6.3±0.7	6.0*±0.7 (92)	6.2±0.6	6.3±0.6	6.0±0.5	5.8*±0.7 (94)
4[b]	10.0±0.9	10.4±1.1	9.9±1.1	9.3±1.2 (93)	9.7±1.0	10.0±1.0	9.6±0.9	9.2±1.3 (95)
4[c]	10.0±0.9	10.4±1.1	9.9±1.1	9.4±1.2 (94)	9.7±1.0	10.0±1.0	9.6±0.9	9.2±1.3 (95)
11	24.3±1.7	24.8±1.8	24.2±2.2	NA	23.7±1.8	24.1±1.7	23.6±2.0	NA
17	38.8±2.4	39.6±2.9	38.6±3.4	NA	37.8±2.2	38.3±2.5	37.5±3.1	NA
21	50.6±3.4	51.7±3.2	50.1±4.8	NA	49.0±3.0	50.1±3.5	48.7±4.2	NA
Body Weight Gain (g)								
1-4	3.6±0.5	3.8±0.6	3.6±0.5	3.1*±0.7 (86)	3.5±0.5	3.7±0.6	3.5±0.5	3.2±0.9 (91)
20-21	3.8±0.5	3.8±0.6	3.4**±0.6 (89)	NA	3.6±0.5	3.7±0.6	3.3±0.6 (92)	NA
4-21	40.5±2.9	41.3±3.0	40.2±4.3	NA	39.3±2.3	40.2±3.1	39.1±3.7	NA

PND = post-natal day; NA= not applicable, dose discontinued
N=23-31
[a] Data obtained from 118-129, MRID 46214401.
[b] Before standardization (culling).
[c] After standardization (culling).
* Statistically significantly different from control, p≤ 0.05
** Statistically significantly different from control, p≤ 0.01.
Number in parentheses is % of control value, calculated by reviewer.

Body weight was measured in male and female pups in subsets III and IV (weeks 0-5 post-weaning), V (weeks 0-1 post-weaning) and VI (weeks 0-6 post-weaning). In subset III, body weight of males at 0.1 mg/kg bw/day was decreased (88-93% of control value); statistical significance was only achieved at week 4. Body weight gain for weeks 0-5 was slightly decreased (94% of control value) in males at 0.1 mg/kg bw/day. These differences are not considered biologically or toxicologically significant. Although there were some decreases at individual time periods, overall post-weaning body weight and body weight gain in the other subsets were not affected. Selected subset VI (chosen for tabulation because observation period was longest of any subset) mean post-weaning offspring body weight data are presented in Table 7.

TABLE 7. Mean (±SD) post-weaning subset VI pup body weight and body weight gain (g) [a]						
Post-weaning Week	Dose (mg/kg bw/day)					
	0	0.03	0.1	0	0.03	0.01
	Males			Females		
BW - Week 0	55.6±7.07	52.6±4.80	56.6±6.18	54.5±5.83	53.5±5.74	51.8±7.50
BW - Week 2	144.5±10.47	138.2±9.52	146.9±7.18	124.5±8.59	124.3±9.49	116.1±15.84
BW - Week 4	228.4±11.38	222.7±12.98	232.2±9.28	161.9±8.85	160.1±11.18	151.2±18.01
BW - Week 6	296.6±15.88	293.2±17.30	305.0±13.31	186.2±9.88	187.6±17.30	177.9±19.94
BWG- Weeks 0-6	241.0±12.92	240.6±15.79	248.4±15.32	131.7±8.71	134.1±15.72	126.1±15.93

[a] Data obtained from pages 152-155, MRID 46214401.
BW= body weight, BWG= body weight gain

3. Developmental landmarks:

a. Sexual maturation: Preputial separation in males occurred between PNDs 41 and 51; the mean age was 43.4, 43.4 and 44.1 days for the control, 0.03 and 0.1 mg/kg bw/day groups, respectively. Vaginal opening occurred between PNDs 28 and 38; the mean age was 31.7, 31.6 and 31.3 days for the control, 0.03 and 0.1 mg/kg bw/day groups, respectively. Body weight at attainment for males and females was similar between the treated and control groups. The data are presented in Table 8.

Table 8. Mean (±SD) age (days) and body weight (g) at sexual maturation [a]			
Parameter	Dose (mg/kg bw/day)		
	0	0.03	0.1
N (M/F)	30/30	29/30	30/30
Preputial separation age body weight	 43.3±1.3 184.5±11.49	 43.4±1.6 189.0±15.89	 44.1±2.4 185.3±15.34
Vaginal opening age body weight	 31.7±1.4 100.9±9.11	 31.6±1.7 102.6±9.86	 31.3±2.2 94.2±11.74

[a] Data obtained from pages 130-131, MRID 46214401.

4. **Behavioral assessment:**

   a. **Functional observational battery (subset IV)**: One male pup in the low-dose group was found dead in week 3 after weaning. There were no treatment-related FOB findings on PNDs 4, 11, 21, 35, 45 or 60. Slight tremors were observed in both male and female pups on PND 4 but the incidence in control and treated groups was comparable.

   b. **Motor/locomotor activity (subset IV):** Total and interval activity data are presented Table 9. Occasional statistically significant increases or decreases in locomotor or rearing activities were noted for single intervals for the treated groups compared with the controls. Habituation was apparent in all groups by PND 21 and both motor and locomotor activities increased as the animals aged. There is an effect at 0.1 mg/kg/day in males and females on PND 21. The increase, over controls, were 38% in males and 21% in females. An increase is also noted at the low dose (0.03 mg/kg/day) in males (9%) and females (10%); however, these increases were determined to be not toxicologically significant due to the lesser magnitude of the effect.

TABLE 9. Interval and total locomotor activity (mean ±SD) [a]

Sub-session		Dose (mg/kg bw/day)					
		Males			Females		
		0	0.03	0.1	0	0.03	0.1
PND 13	1	464.1±157.0	435.1±170.8	429.0±102.0	354.3±78.0	430.4±141.0	435.0±210.9
	2	285.0±181.6	276.6±178.4	233.8±186.8	332.3±305.9	273.9±217.5	229.2±249.3
	3	280.0±164.0	241.3±242.1	271.1±147.5	382.5±457.8	180.4±140.5	276.4±341.0
	4	282.6±161.8	183.9±209.1	239.7±147.0	349.1±383.0	366.0±428.0	347.5±400.0
	5	171.1±134.5	227.0±257.2	170.4±215.8	330.3±361.4	459.0±500.6	214.7±214.0
	6	85.0±65.5	296.5±256.4	82.9±68.7	172.2±257.4	283.3±233.6	284.9±284.6
	7	113.3±118.5	342.8±327.0	68.1±54.5	247.9±474.5	118.9±119.4	285.3±391.0
	8	127.4±161.0	136.4±125.2	45.5±30.3	139.5±150.4	221.7±446.9	149.8±264.5
	9	172.3±235.5	176.7±331.3	76.9±134.8	45.8±42.9	177.4±320.9	236.4±327.8
	10	115.8±134.0	62.2±79.3	179.6±470.0	101.1±208.0	247.3±488.2	139.4±214.7
	11	141.5±230.2	50.4±40.2	116.0±147.1	118.9±217.0	348.3±560.7	115.7±154.0
	12	76.2±144.9	85.6±104.1	171.5±408.9	325.0±612.8	222.1±349.5	443.1±728.1
	Sum 1-12	2314.1± 821.4	2514.4± 1108.8	2085.0± 1353.1	2898.5± 2708.5	3328.7± 2868.6	3157.3± 2751.4
PND 17	1	603.1±285.5	589.6±271.1	795.9±239.2	795.4±316.3	680.8±274.7	664.4±238.9
	2	427.0±298.4	486.0±283.2	596.1±240.9	568.5±211.3	481.9±287.0	770.5±248.6
	3	447.8±370.1	434.3±419.6	512.0±352.0	347.9±274.4	284.1±256.6	547.8±326.9
	4	383.1±317.0	263.1±419.2	259.8±239.8	229.7±250.9	186.9±234.0	338.8±327.0
	5	262.3±362.0	211.0±357.3	140.8±164.6	321.7±397.9	112.1±254.0	235.9±335.6
	6	209.9±248.3	176.9±244.9	434.5±423.9	175.1±325.9	37.9±39.0	157.8±238.6
	7	229.3±315.1	194.5±373.6	110.5±101.0	35.3±31.6	15.3±15.5	125.9±164.8
	8	216.6±371.4	77.0±165.6	89.0±160.5	58.6±107.4	16.3±16.7	202.2±239.0
	9	83.3±98.4	120.0±284.7	185.7±323.9	213.0±306.8	44.9±73.7	168.9±275.1
	10	194.3±251.6	161.2±420.0	152.8±245.8	215.5±315.5	62.8±131.2	117.1±196.6
	11	273.8±314.4	98.0±232.1	145.3±212.4	233.2±230.1	149.0±200.9	58.8±62.9
	12	420.8±415.4	205.4±380.3	122.5±199.5	254.8±414.8	177.1±214.3	108.9±250.1
	Sum 1-12	3751.1± 1861.4	3016.9± 2559.8	3544.9± 1776.1	3448.6± 2046.0	2249.1± 1137.3	3496.8± 1307.4
PND 21	1	1126.0± 232.5	1216.9± 279.9	1317.0± 258.9	1203.2± 175.8	1325.2± 287.0	1338.0± 300.8
	2	460.3±157.2	558.3±231.8	646.3±180.7	587.8±237.4	617.7±324.2	590.5±164.0
	3	350.4±216.3	399.1±200.5	445.8±191.8	416.3±269.1	409.5±318.0	541.8±122.8
	4	196.1±225.7	284.9±265.5	496.0*±296.2	290.2±222.3	392.4±359.1	381.6±176.2
	5	154.0±169.3	186.7±264.9	389.1*±242.7	237.8±237.3	257.5±303.3	219.1±168.0
	6	135.4±198.3	146.1±213.5	356.2±296.4	196.3±297.7	116.3±146.6	286.8±316.6
	7	83.8±141.7	159.6±230.9	153.8±139.1	82.4±99.0	44.0±55.5	156.6±120.1
	8	93.3±151.6	119.1±186.2	202.3±189.6	86.0±211.3	155.1±293.9	166.1±302.6
	9	124.5±196.7	106.6±172.9	143.2±249.9	75.2±165.3	104.4±259.5	91.3±137.9
	10	185.7±322.2	117.1±173.5	56.4±68.1	113.2±287.9	92.4±231.8	89.0±201.5
	11	99.5±132.9	52.2±47.8	103.1±225.1	34.1±30.5	75.0±163.9	58.2±97.9
	12	131.6±208.6	67.8±126.1	39.3±67.7	36.2±37.4	96.6±255.8	149.8±218.3
	Sum 1-12	3140.7± 1429.4	3414.5± 1652.0	4348.6± 1203.0	3358.7± 1742.3	3685.9± 2691.2	4068.7± 783.5

TABLE 9. Interval and total locomotor activity (mean ±SD) [a]

Sub-session		Dose (mg/kg bw/day)					
		Males			Females		
		0	0.03	0.1	0	0.03	0.1
PND 60	1	1249.4± 181.4	1276.0± 172.7	1340.4± 158.1	1601.9± 184.4	1592.8± 243.2	1594.0± 277.9
	2	1068.5± 235.5	1079.8± 217.0	1100.5± 160.2	1135.2± 169.2	1133.6± 188.8	1204.5± 245.9
	3	961.9±293.4	918.3±150.5	922.7±190.1	1020.4±213.0	979.8±138.0	1012.5±204.6
	4	779.3±313.0	678.2±102.0	863.5±173.2	866.6±211.8	819.3±310.9	943.0±252.3
	5	638.4±318.9	559.2±207.9	658.0±212.4	808.0±327.5	796.1±337.0	879.5±270.0
	6	761.2±361.4	655.9±262.0	706.7±179.6	943.0±203.5	695.2±227.8	755.9±119.2
	7	710.4±369.3	766.5±227.2	579.3±208.9	716.3±157.8	771.4±197.6	703.2±276.4
	8	530.6±183.6	510.5±129.3	697.3±217.5	757.0±222.4	624.5±388.5	643.6±273.4
	9	602.7±318.4	528.2±147.1	527.3±204.3	781.6±362.8	706.0±336.9	633.5±304.9
	10	571.7±407.0	483.2±366.1	630.6±203.2	565.7±346.9	631.8±282.1	590.0±372.7
	11	422.3±275.2	486.1±324.0	431.9±304.4	546.4±331.6	553.7±269.0	370.2±408.3
	12	336.1±198.6	405.1±240.4	300.2±304.8	775.8±394.3	583.9±387.7	445.4±448.9
	Sum 1-12	8632.5± 2526.0	8347.2± 1325.2	8758.4± 1443.9	10518± 2253.4	9888.0± 1688.9	9775.2± 1780.4

[a] Data obtained from pages 229-244, MRID 46214401
N =9-10
* Statistically different from control, $p<0.05$

TABLE 10. Motor activity (rearing) - intervals and total (mean ±SD) [a]

Sub-session		Dose (mg/kg bw/day)					
		Males			Females		
		0	0.03	0.1	0	0.03	0.1
PND 13	1	13.8±9.4	15.9±11.4	14.7±9.6	15.6±8.3	20.0±9.5	19.3±13.7
	2	10.2±9.1	13.2±7.9	8.8±9.1	13.5±16.0	14.8±15.9	11.1±13.6
	3	14.4±10.2	10.3±12.3	13.3±12.7	9.2±10.7	9.0±12.2	12.5±13.1
	4	12.6±11.3	6.1±7.7	7.4±7.0	9.4±7.3	11.5±14.1	10.9±9.0
	5	7.7±9.6	6.0±4.3	4.5±8.9	8.8±9.5	16.7±13.3	7.3±9.0
	6	0.9±0.9	7.6±6.8	0.9±1.9	4.2±6.0	11.5±9.4	9.4±9.2
	7	5.3±6.4	10.4±13.8	0.3±0.7	6.6±11.7	3.3±6.2	9.6±12.0
	8	3.2±5.1	6.1±7.8	0.2±0.6	5.9±11.7	2.8±5.7	3.0±4.8
	9	3.6±6.9	6.0±12.2	0.4±0.7	0.6±1.9	3.5±7.4	5.0±8.0
	10	2.8±5.9	1.5±3.7	1.8±4.7	1.9±6.0	4.7±9.6	3.9±8.6
	11	5.1±11.1	0.7±1.1	1.8±3.5	1.2±3.8	7.3±12.7	2.3±5.3
	12	1.6±4.3	2.0±3.7	1.5±4.4	6.2±14.2	6.0±12.1	7.6±13.4
	Sum 1-12	81.1±47.4	85.8±40.9	55.6±32.2	83.1±64.2	111.1±58.6	101.9±79.1
PND 17	1	26.4±9.4	29.3±13.1	35.4±7.2	29.0±4.8	33.2±15.0	31.0±8.7
	2	22.8±13.6	24.8±12.4	28.8±13.0	28.8±11.9	24.7±13.0	34.6±13.7
	3	19.4±14.3	18.5±15.0	22.3±14.3	18.0±14.1	14.9±12.2	24.7±14.5
	4	16.5±13.4	9.2±14.2	11.6±10.6	12.4±13.2	10.4±12.4	17.4±14.5
	5	8.5±14.3	8.4±12.6	7.5±8.1	12.7±15.1	4.3±9.4	7.0±14.5
	6	8.9±13.2	5.0±7.5	12.6±15.5	5.3±8.3	1.7±3.8	3.9±7.6
	7	10.7±16.8	4.9±11.9	2.6±3.9	0.4±0.7	1.0±2.8	5.6±8.3
	8	8.2±16.2	1.8±5.3	0.9±1.7	1.4±3.0	0.5±1.1	6.8±9.6
	9	2.5±5.1	2.6±6.9	5.4±11.4	7.3±11.9	1.5±3.1	4.8±9.0
	10	5.1±8.6	4.6±12.9	5.1±9.6	9.5±15.7	1.2±3.8	2.9±7.8
	11	10.7±16.5	3.0±9.5	6.6±10.2	8.9±9.6	4.1±6.3	0.8±1.5
	12	9.8±10.3	2.8±6.5	3.3±6.2	5.3±9.6	4.7±7.3	3.1±6.6
	Sum 1-12	149.5±89.4	114.9±84.3	142.1±68.8	139.0±89.3	102.2±48.3	142.6±57.5
PND 21	1	37.2±11.6	35.6±15.5	42.0±12.9	38.0±10.2	40.4±11.4	44.7±11.9
	2	21.3±6.3	24.6±14.2	29.0±5.6	20.7±9.5	23.5±14.5	27.5±8.8
	3	16.3±8.6	16.7±9.5	20.0±7.6	20.3±14.0	14.7±10.0	18.5±8.4
	4	7.7±9.7	9.2±8.7	21.1*±9.6	11.7±9.8	14.8±11.9	14.5±9.8
	5	6.1±8.3	6.5±8.5	16.7*±12.5	7.0±6.5	8.7±10.5	7.8±11.8
	6	3.9±6.1	4.4±9.5	13.4±10.7	4.9±7.3	3.6±5.7	9.9±10.7
	7	3.2±5.6	6.1±10.8	4.4±5.7	2.1±3.9	1.5±1.8	5.2*±3.6
	8	2.9±6.2	2.6±5.2	7.0±7.9	1.7±5.0	6.0±11.4	4.8±9.2
	9	3.1±6.0	3.1±5.6	4.9±10.3	1.5±4.7	3.1±9.1	3.4±6.9
	10	6.6±14.0	2.0±4.1	1.4±3.8	2.5±7.9	2.0±6.3	3.3±8.0
	11	2.7±4.7	0.8±1.8	2.1±6.6	0.7±2.2	1.7±5.0	2.1±4.6
	12	4.3±8.0	1.7±3.7	0.5±1.6	0.7±1.9	3.0±9.5	4.7±7.6
	Sum 1-12	115.3±54.4	113.3±59.5	162.5±48.2	111.8±60.0	123.0±91.9	146.4±46.3

TABLE 10. Motor activity (rearing) - intervals and total (mean ±SD) [a]							
Sub-session		Dose (mg/kg bw/day)					
		Males			Females		
		0	0.03	0.1	0	0.03	0.1
PND 60	1	35.4±5.3	37.0±9.3	34.5±7.2	40.9±11.8	45.0±10.2	44.7±12.6
	2	30.0±5.5	31.7±8.9	31.8±6.5	29.3±10.0	30.4±9.3	36.2±12.0
	3	25.3±6.3	23.7±7.5	26.1±2.7	25.2±9.5	29.5±7.9	29.0±5.5
	4	20.8±10.3	21.1±8.9	23.0±5.9	20.3±10.5	20.8±10.7	25.8±8.2
	5	15.1±8.5	15.3±6.7	17.1±6.8	21.0±14.2	21.0±12.6	21.3±11.6
	6	15.7±9.3	13.9±8.8	18.9±9.2	23.9±10.1	15.9±8.8	17.4±9.3
	7	16.3±9.9	16.4±4.7	15.0±9.0	13.8±6.8	19.5±10.1	18.8±10.0
	8	8.7±4.2	11.9±3.4	16.3*±7.2	14.4±8.2	11.1±7.8	16.2±8.2
	9	12.3±7.9	11.4±4.0	12.7±7.5	19.4±12.9	11.8±7.2	14.2±8.2
	10	9.0±4.8	10.4±9.3	13.5±5.3	13.0±9.0	14.4±11.0	8.1±7.4
	11	7.3±5.8	8.8±6.6	7.4±5.2	11.3±8.4	11.1±7.8	8.0±12.1
	12	5.6±6.3	7.9±6.1	6.0±7.9	12.0±9.1	12.0±8.9	7.9±8.4
	Sum 1-12	201.5±41.7	209.6±37.6	222.3±49.9	244.7±90.3	242.5±65.1	247.6±68.9

[a] Data obtained from pages 245-260 in the study report, MRID 46214401
N =9-10
* Statistically different from control, p<0.05

c. **Auditory startle reflex habituation (subset III) :** The amplitude and latency data are presented in Tables 11 and 12, respectively. A clear effect is seen at 0.1 mg/kg/day in males on PND 60 which is supported by a smaller effect in the same direction seen in males on PND 24 and by habituation data. The effect is consistent across blocks. Data from the follow-up study at 0.2 mg/kg/day support the results seen at 0.1 mg/kg/.day. No differences in latency to peak response were observed between the treated and control groups of either sex on either testing day. Habituation was apparent on both testing days in both sexes.

TABLE 11. Auditory startle amplitude (v) (mean ±SD) [a]

	Trial Block	Dose (mg/kg bw/day)		
		0	0.03	0.1
Males				
PND 24	1	519.3±205.1	485.7±175.4	407.6±196.1
	2	374.2±89.9	464.1±170.0	310.8**±155.6
	3	352.6±69.9	410.1±183.3	308.7±92.6
	4	353.3±106.8	374.1±87.3	305.4±66.5
	5	350.2±86.9	391.3±101.2	309.9±95.5
	Mean	389.9±99.1	425.1±116.7	328.5±89.9
PND 60	1	1447.6±544.0	1473.4±791.6	1204.8±368.2
	2	929.3±196.2	1178.8±669.9	619.4**±190.8
	3	923.4±430.1	824.5±520.8	521.9**±212.9
	4	857.3±524.4	729.0±358.2	561.8±260.8
	5	797.9±418.7	760.8±488.3	439.9*±157.0
	Mean	991.1±306.2	993.3±528.5	669.5±183.6
Females				
PND 24	1	426.0±141.2	435.2±155.8	450.3±163.8
	2	375.1±143.6	433.5±199.0	503.9±162.4
	3	352.3±99.8	402.4±149.4	433.8±106.6
	4	337.2±74.4	427.7±148.5	441.6±125.7
	5	338.6±89.1	371.0±124.0	420.6±119.7
	Mean	365.8±93.8	414.0±140.9	450.1±108.2
PND 60	1	1055.3±530.6	1031.1±501.5	1094.1±830.0
	2	942.3±436.3	1055.0±811.6	756.4±360.5
	3	747.9±267.1	763.8±618.9	558.0±170.6
	4	536.3±227.4	714.5±365.9	613.8±397.9
	5	535.5±191.0	470.5±242.1	450.9±196.2
	Mean	763.4±265.7	807.0±392.5	694.6±346.4

[a]Data obtained from 261-264, MRID 46214401.
N=9-10
* Statistically significantly different from control value, $p<0.05$
** Statistically significantly different from control value, $p<0.01$

TABLE 12. Auditory startle reflex habituation: time to peak amplitude (mean msec ±S.D.) [a]

	Trial Block	Dose (ppm)		
		0	0.03	0.1
Males				
PND 24	1	36.6±7.1	34.9±8.9	37.4±8.8
	2	34.3±8.0	33.3±10.5	33.7±8.4
	3	30.0±5.7	30.3±8.2	32.3±4.9
	4	29.8±6.0	31.6±4.6	30.6±6.9
	5	28.7±7.2	26.8±4.8	32.4±6.6
	Mean	31.9±5.4	31.4±6.1	33.3±4.4
PND 60	1	43.5±13.4	42.2±13.3	42.8±10.2
	2	30.6±6.0	40.2±12.3	31.6±5.8
	3	33.1±8.9	35.1±11.0	29.3±6.3
	4	30.1±7.5	31.9±4.1	28.5±6.0
	5	33.0±11.7	28.7±5.2	29.9±9.2
	Mean	34.1±7.3	35.6±7.7	32.4±4.0
Females				
PND 24	1	35.8±11.1	32.7±8.3	36.2±8.3
	2	29.1±6.8	31.4±7.7	34.7±7.9
	3	28.9±8.2	30.3±5.8	29.4±5.2
	4	27.0±6.5	28.1±5.8	30.6±5.9
	5	27.5±9.9	28.6±6.8	26.6±5.8
	Mean	29.7±6.8	30.2±5.1	31.5±4.5
PND 60	1	37.8±10.0	39.8±10.5	41.7±12.1
	2	36.1±11.4	43.0±12.4	35.2±9.3
	3	31.7±6.4	34.3±12.3	29.7±6.1
	4	27.9±4.6	35.5±11.8	30.6±7.8
	5	28.5±6.2	28.1±6.5	27.8±4.6
	Mean	32.4±5.7	36.1±7.2	33.0±6.2

[a]Data obtained from pages 265-268, MRID 46214401. N=10

d. **Learning and memory testing (water maze, subsets V and VI):** On PND 23, a significantly decreased number of successful males at 0.03 and 0.1 mg/kg bw/day was observed in the last trial. Also on PND 23, a significant increase in the mean time to escape was observed in females at 0.03 and 0.1 mg/kg bw/day in learning trial 3. On PND 60, males at 0.03 mg/kg bw/day had a significantly increased mean time to escape in learning trial 3. The data are presented in Tables 13 (PND 23 and 30) and 14 (PND 60 and 67). Learning was occurred during both testing sessions as an increase in the number of animals with successful trials and a decrease in the time to completion over successive trials.

TABLE 13. Water maze performance in PND 23 and 30 offspring [a]

Session/parameter		Dose (mg/kg bw/day)		
		0	0.03	0.1
Males				
Number (%) Animals Reaching Criteria				
Learning 1 (PND 23)	Trial 2	5 (50)	4 (40)	5 (50)
	Trial 3	6 (60)	6 (60)	5 (50)
	Trial 4	6 (60)	6 (60)	8 (80)
	Trial 5	6 (60)	8 (80)	9 (90)
	Trial 6	9 (90)	9 (90)	9 (90)
Memory (PND 30)		7 (70)	9 (90)	8 (80)
Learning 2 (PND 30)	Trial 2	2 (20)	5 (50)	3 (30)
	Trial 3	5 (50)	4 (40)	2 (20)
	Trial 4	7 (70)	5 (50)	3 (30)
	Trial 5	8 (80)	5 (50)	5 (50)
	Trial 6	10 (100)	6* (60)	6* (60)
Time (sec ± SD)				
Learning 1 (PND 23)	Trial 2	19±15.8	27±26.1	36±38.6
	Trial 3	14±9.9	17±11.7	15±5.6
	Trial 4	18±11.8	17±15.0	12±11.3
	Trial 5	12±11.9	8±2.9	8±4.1
	Trial 6	8±4.5	8±2.5	6±2.6
Memory (PND 30)		7±4.4	6±3.1	8±4.8
Learning 2 (PND 30)	Trial 2	19±12.1	16±10.8	20±17.9
	Trial 3	13±10.4	14±6.4	15±11.4
	Trial 4	10±9.0	11±6.3	14±12.3
	Trial 5	6±5.6	8±5.1	9±5.9
	Trial 6	5±2.8	8±5.3	7±5.2
Females				
Number (%) Animals Reaching Criteria				
Learning 1 (PND 23)	Trial 2	5 (50)	3 (30)	4 (40)
	Trial 3	7 (70)	3 (30)	6 (60)
	Trial 4	9 (90)	7 (70)	9 (90)
	Trial 5	9 (90)	9 (90)	10 (100)
	Trial 6	10 (100)	9 (90)	9 (90)
Memory (PND 30)		8 (80)	8 (80)	8 (80)
Learning 2 (PND 30)	Trial 2	1 (10)	2 (20)	1 (10)
	Trial 3	3 (30)	4 (40)	4 (40)
	Trial 4	2 (20)	3 (30)	3 (30)
	Trial 5	3 (30)	6 (60)	6 (60)
	Trial 6	3 (30)	5 (50)	6 (60)
Time (sec ± SD)				
Learning 1 (PND 23)	Trial 2	21±10.5	27±19.4	18±8.0
	Trial 3	11±17.9	25*±13.2	18*±11.0
	Trial 4	8±9.7	14±7.8	7±2.1
	Trial 5	6±5.2	11±12.3	5±0.9
	Trial 6	7±9.7	6±3.5	5±2.2

TABLE 13. Water maze performance in PND 23 and 30 offspring [a]

Session/parameter		Dose (mg/kg bw/day)		
		0	0.03	0.1
Memory (PND 30)		7±5.9	8±5.0	6±2.3
Learning 2 (PND 30)	Trial 2	22±18.3	26±16.7	17±7.6
	Trial 3	15±6.4	17±14.2	12±6.9
	Trial 4	12±5.7	15±8.8	13±10.2
	Trial 5	10±5.2	8±5.1	8±4.8
	Trial 6	10±5.5	9±5.5	9±8.7

[a] Data obtained from pages 156-159, MRID 46214401.
* Statistically different from control, $p<0.05$
N=10

TABLE 14. Water maze performance in PND 60 and 67 offspring [a]

Session/parameter		Dose (mg/kg bw/day)		
		0	0.03	0.1
Males				
Number (%) Animals Reaching Criteria				
Learning 1 (PND 60)	Trial 2	6 (60)	7 (70)	6 (60)
	Trial 3	8 (80)	6 (60)	8 (80)
	Trial 4	9 (90)	10 (100)	10 (100)
	Trial 5	9 (90)	10 (100)	10 (100)
	Trial 6	10 (100)	10 (100)	10 (100)
Memory (PND 67)		10 (100)	10 (100)	10 (100)
Learning 2 (PND 67)	Trial 2	2 (20)	1 (10)	0 (0)
	Trial 3	6 (60)	3 (30)	3 (30)
	Trial 4	5 (50)	7 (70)	5 (50)
	Trial 5	5 (50)	7 (70)	7 (70)
	Trial 6	7 (70)	10 (100)	8 (80)
Time (sec ± SD)				
Learning 1 (PND 60)	Trial 2	13±6.1	15±8.1	16±10.7
	Trial 3	7±4.4	13*±7.0	8±4.5
	Trial 4	7±5.7	5±2.3	4±2.0
	Trial 5	6±6.7	5±1.8	4±1.4
	Trial 6	3±1.1	5±2.8	3±0.8
Memory (PND 67)		5±2.0	8±9.0	5±3.3
Learning 2 (PND 67)	Trial 2	17±7.9	19±9.9	19±10.2
	Trial 3	13±16.2	12±5.9	17±16.5
	Trial 4	10±6.5	7±5.5	11±10.1
	Trial 5	10±7.5	8±6.3	7±4.6
	Trial 6	9±7.4	5±3.2	5±3.9
Females				
Number (%) Animals Reaching Criteria				
Learning 1 (PND 60)	Trial 2	7 (70)	7 (70)	7 (70)
	Trial 3	6 (60)	8 (80)	7 (70)
	Trial 4	8 (80)	9 (90)	8 (80)
	Trial 5	6 (60)	9 (90)	7 (70)
	Trial 6	9 (90)	9 (90)	8 (80)
Memory (PND 67)		9 (90)	9 (90)	8 (80)
Learning 2 (PND 67)	Trial 2	0 (0)	1 (10)	3 (30)
	Trial 3	3 (30)	3 (30)	5 (50)
	Trial 4	3 (30)	3 (30)	3 (30)
	Trial 5	6 (60)	5 (50)	3 (30)
	Trial 6	5 (50)	5 (50)	4 (40)
Time (sec ± SD)				
Learning 1 (PND 60)	Trial 2	12±4.7	12±4.4	12±8.2
	Trial 3	15±20.4	8±5.2	11±10.3
	Trial 4	10±9.5	6±3.0	7±3.3
	Trial 5	9±8.6	6±8.1	9±6.9
	Trial 6	6±4.2	5±2.9	7±5.5

TABLE 14. Water maze performance in PND 60 and 67 offspring [a]				
Session/parameter		Dose (mg/kg bw/day)		
		0	0.03	0.1
Memory (PND 67)		5±3.6	5±2.3	10±8.6
Learning 2 (PND 67)	Trial 2	16±7.3	17±10.3	10±4.1
	Trial 3	8±2.7	8±3.2	8±3.6
	Trial 4	8±3.2	8±4.1	11±8.5
	Trial 5	8±4.5	6±4.2	10±7.0
	Trial 6	7±3.1	6±3.7	11±10.5

[a] Data obtained from pages 160-163, MRID 46214401.
* Statistically different from control, p<0.05 N=10

5. **Cholinesterase activity:** Results of the cholinesterase (ChE) activity assessment are presented in Table 15. PND 4 male offspring at 0.3/0.2 mg/kg bw/day had RBC ChE activity inhibited by 23%. There were no other treatment-related significant ChE changes in males or females on PND 4.

On PND 21 male pups at 0.1 mg/kg bw/day, had inhibition of ChE activity by 15% in serum, 16% in RBC, and by 21% in brain. Only serum ChE activity was inhibited (19% inhibition) in females at 0.1 mg/kg bw/day.

TABLE 15. Cholinesterase activity in dams and offspring				
Cholinesterase activity (mean ± SD)	Dose (mg/kg bw/day)			
	0	0.03	0.1	0.3/0.2
Lactation day 21 dams				
Serum (µkat/L)	20.3 ±3.9	17.9± 4.2	19.3±3.6	NA
RBC (µkat/L)	34.5±2.8	33.3±4.1	33.3±3.2	
Brain (µkat/g)	2.2 ± 0.9	2.1±0.6	3.2*±1.0	
Day 4 male offspring				
Serum (µkat/L)	13.3±0.9	13.2± 1.3	12.8±0.9	12.9± 1.0
RBC (µkat/L)	12.3±2.1	12.2±3.6	13.9±3.0	9.5*±2.2 (23)
Brain (µkat/g)	1.2±0.1	1.2±0.1	1.2±0.1	1.1±0.1
Day 4 female offspring				
Serum (µkat/L)	13.4±0.9	13.4± 0.7	12.9±1.1	13.6±1.0
RBC (µkat/L)	10.1±2.1	11.1±2.6	12.1±1.8	11.4±2.2
Brain (µkat/g)	1.2±0.1	1.2±0.1	1.2±0.1	1.1±0.1
Day 21 male offspring				
Serum (µkat/L)	13.9 ±1.7	13.9±1.9	11.8*±1.4 (15)	NA
RBC (µkat/L)	44.3±5.2	45.4±5.4	37.1**±5.1 (16)	
Brain (µkat/g)	1.4±0.3	1.3±0.1	1.1**±0.1 (21)	
Day 21 female offspring				
Serum (µkat/L)	13.8±1.4	13.9±1.3	11.2**±1.7 (19)	NA
RBC (µkat/L)	41.1±6.1	41.4±3.4	39.1±4.2	
Brain (µkat/g)	1.4±0.3	1.4±0.3	1.4±0.3	

Data obtained from pages 293-297, MRID 46214401.
N=10
NA= not applicable, dose discontinued.
* Statistically different from control, p<0.05
** Statistically different from control, p<0.01
Number in parantheses is % inhibition relative to control; calculated by reviewer

6. Postmortem results (subsets II and III):

a. **Brain weights:** Mean brain weight data are presented in Table 16. No treatment-related effects were noted on Day 22. On PND 60±2, absolute weight was slightly but significantly decreased (96% of control value) in females at 0.1 mg/kg bw/day.

TABLE 16. Mean (±SD) Brain Weight Data in Offspring [a]

Parameter	Dose (mg/kg bw/day)		
	0	0.03	0.1
Males			
Day 22			
Terminal body weight (g)	51.59±3.22	53.97±6.36	52.30±7.33
Brain weight (g)	1.61±0.06	1.59±0.08	1.58±0.07
Day 60±2			
Terminal body weight (g)	283.86±14.81	284.94±20.09	266.79±21.79
Brain weight (g)	1.96±0.06	1.99±0.11	1.97±0.06
Females			
Day 22			
Terminal body weight (g)	51.40±2.62	54.52±3.82	50.49±4.29
Brain weight (g)	1.56±0.05	1.59±0.06	1.57±0.04
Day 60±2			
Terminal body weight (g)	183.14±12.12	185.06±11.89	180.25±9.8
Brain weight (g)	1.88±0.06	1.87±0.09	1.81*±0.06 (96)

[a] Data obtained from pages 298-305, MRID 46214401
N=10
* Statistically significantly different from control value, $p<0.05$

b. **Macroscopic examination:** No treatment-related effects were reported for male or female offspring on PND 22 or 60±2.

c. **Neuropathology (subsets II and III)**

1) **Microscopic examination:** No treatment-related effects were reported for male or female offspring on PNDs 22 and 60±2.

2) **Brain Morphometry:** Morphometric evaluation, presented in Table 17 revealed the following: a statistically significant decrease (99% of control value) in the cerebrum width in females at 0.1 mg/kg bw/day on PND 60±2 and a significant decrease (95% of control value) in the neocortex (FC right) in females at 0.03 mg/kg bw/day on PND 22.

TABLE 17. Mean (±SD) brain morphometric data [a]

Parameter	Dose (mg/kg bw/day)		
	0	0.03	0.1
Males			
Day 22			
Cerebrum			
Length (cm)	1.37±0.02	1.34±0.04	1.35±0.03
Width (cm)	1.47±0.03	1.46±0.02	1.45±0.03
Cerebellum			
Length (cm)	0.73±0.03	0.72±0.03	0.73±0.03
Width (cm)	1.10±0.03	1.08±0.04	1.08±0.05
FC Left (μm)	1493±62	1533±82	1516±63
PC Left (μm)	1590±56	1587±68	1582±77
Termination			
Cerebrum			
Length (cm)	1.49±0.02	1.48±0.05	1.49±0.04
Width (cm)	1.52±0.04	1.53±0.02	1.52±0.02
Cerebellum			
Length (cm)	0.75±0.03	0.75±0.03	0.75±0.02
Width (cm)	1.17±0.03	1.16±0.05	1.18±0.03
Females			
Day 22			
Cerebrum			
Length (cm)	1.35±0.03	1.35±0.03	1.35±0.03
Width (cm)	1.44±0.03	1.44±0.02	1.44±0.02
Cerebellum			
Length (cm)	0.72±0.03	0.72±0.03	0.70±0.02
Width (cm)	1.08±0.03	1.08±0.04	1.08±0.03
FC Right (μm)	1578±50	1492**±59	1536±105
Folpyr half (μm)	306±31	303±20	300±31
Termination			
Cerebrum			
Length (cm)	1.45±0.04	1.45±0.03	1.44±0.03
Width (cm)	1.49±0.02	1.49±0.02	1.47**±0.02
Cerebellum			
Length (cm)	0.75±0.03	0.75±0.03	0.74±0.01
Width (cm)	1.16±0.02	1.15±0.04	1.15±0.03

[a] Data obtained from pages 309-314 in the study report, MRID 46214401.
FC Left = frontal cortex, left brain; FC right = frontal cortex, right brain; PC Left = parietal cortex, left brain; Folpyr half = folium pyramis of cerebellum, half way between tip and base
N = 10
** Significantly different from control, $p<0.01$

NOTE: **The results of the supplemental study (MRID No. 46214402) conducted with a single oral dose (0.2 mg/kg/day) are discussed below:**

B. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No animals died or had clinical signs of toxicity in the control and treated dams, including during the FOB examinations.

2. **Body weight and food consumption**: Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 18. Mean body weights in the treated group were comparable to controls during gestation and lactation. Mean body weight gain during gestation and lactation was unaffected by treatment but was significantly decreased (26% of control value) on LDs 9-10.

   Food consumption in treated animals was comparable to controls during gestation and lactation.

TABLE 18. Selected mean (±SD) maternal body weight and food consumption[a]

Observations/study interval	Dose (mg/kg bw/day)	
	0	0.2
Gestation (n= 31-34)		
Body wt. Gestation day 0 (g)	162.7±10.2	163.9±7.7
Body wt. Gestation day 7 (g)	194.0±13.6	193.3±9.6
Body wt. Gestation day 15 (g)	227.8±17.4	225.5±12.3
Body wt. Gestation day 20 (g)	271.3±20.5	270.0±15.5
Wt. gain gestation days 6-20 (g)	80.2±11.3	79.1±8.9
Food consumption gestation days 0-6 (g/animal/day)	16.7±1.6	16.5±1.2
Food consumption gestation days 6-20 (g/animal/day	17.6±0.7	17.3±0.8
Lactation (n=31-41)		
Body wt. lactation day 0 (g)	209.3±16.2	209.4±13.8
Body wt. lactation day 9 (g)	236.9±18.3	237.0±13.4
Body wt. lactation day 10 (g)	243.7±20.6	238.8±14.8
Body wt. lactation day 21 (g)	241.2±16.5	237.2±14.0
Wt gain lactation days 0-1 (g)	-0.4±9.6	-1.1±8.1
Wt gain lactation days 1-2 (g)	5.8±5.5	3.5±4.7
Wt. gain lactation days 9-10	6.8±8.0	1.8**±5.9 (26)
Wt gain lactation days 0-21(g)	32.8±10.0	28.7±12.0
Food consumption lactation days 1-21 (g/animal/day)	43.5±13.7	41.9±11.9

[a]*Data obtained from pages 98-107, MRID 46214402*

** Statistically significantly different from control, p≤ 0.01.

Number in parentheses is % of control value, calculated by reviewer.

3. **Reproductive performance**: Six control and four treated females failed to deliver pups. The fertility index was 87% and 91% for the control and treated groups, respectively. The mean duration of gestation was 22.1 days in both control and treated groups. The gestation index was 97% and 100% in the control and treated groups, respectively. Results for the maternal animals are summarized from the report in Table 19.

TABLE 19. Reproductive performance [a]

Observation	Dose (mg/kg bw/day)	
	0	0.2
Number mated	45	45
Number pregnant	39	41
Fertility index (%)	87	91
Intercurrent deaths	0	0
Gestation index (%)	97	100
Mean (±SD) gestation duration (days)	22.1±0.5	22.1±0.5
Incidence of dystocia	0	0

[a] Data obtained from page108, MRID 46214402.

4. **Cholinesterase activity**: Serum and RBC ChE were significantly inhibited by 21% and 13%, respectively, on PND 21 in treated animals. Brain ChE was inhibited by 17% (n.s.). The data are included with offspring data in Table 29.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized from the report in the Table 20. The mean number of pups/dam, the rate of live born, stillborn and dead pups and pup viability and mortality were not affected by treatment. The sex ratio of live pups on the day of birth and PND 21 also was not affected by treatment. There were no treatment-related clinical signs of toxicity.

TABLE 20. Litter size and viability [a]

Observation	Dose (mg/kg bw/day)	
	0	0.2
Total number born	331	370
Pups/dam delivered	8.5±2.2	9.0±1.3
Number of litters	39	41
Number with live born litters	38	41
Number born live	327	366
Number with stillborn pups	3	3
Number with all stillborn pups	1	0
Number born dead	4	4
Sex Ratio Day 0 (% ♂)	47.7	50.8
# Deaths Days 1-4 (%)	2 (0.6)	2 (0.5)
# Deaths Days 5-7 (%)	0	0
# Deaths Days 8-14	0	3 (0.8)
# Deaths Days 15-21	3 (0.9)	0
Mean litter size:		
Day 0	8.4±2.4	8.9±1.4
Day 4 (pre-cull)	8.1±2.8	8.6±2.0
Day 4 (post-cull)	6.4±3.3	6.6±3.1
Day 11	6.4±3.3	6.6±3.1
Day 17	5.8±3.0	6.1±2.8
Day 21	5.7±3.0	6.1±2.8
Live birth index (%)[b]	99	99

[a] Data obtained from pages 109-111, MRID 46214402.
[b] Calculated by the reviewer.

2. **Body weight:** There was no treatment-related effect on body weight during lactation. Body weight gain was significantly decreased in treated male pups during lactation days 16-17 (92% of control value) and lactation days 19-20 and 20-21 (91-92% of control value); however, the overall body weight gain during lactation was comparable to the control value. Selected mean preweaning pup body weight data are presented in Table 21.

TABLE 21. Selected mean (±SD) pre-weaning pup body weight and body weight gain [a]

PND	Dose (mg/kg bw/day)			
	0	0.2	0	0.2
	Males		Females	
Body Weight (g)				
1	6.7±0.5	6.8±0.4	6.4±0.6	6.5±0.5
4[b] (pre-cull)	10.3±0.9	10.3±0.8	10.0±1.0	10.0±0.8
4[c] (post-cull)	10.4±0.9	10.3±0.7	10.0±0.9	10.0±0.9
11	23.8±2.2	24.0±1.4	23.2±2.2	23.3±1.6
21	50.1±4.6	48.8±3.4	48.6±4.3	47.8±3.4
Body Weight Gain (g)				
1-4	3.6±0.5	3.5±0.4	3.6±0.5	3.5±0.4
16-17	2.4±0.5	2.2*±0.3 (92)	2.3±0.5	2.1±0.3
19-20	3.2±0.4	2.9**±0.4 (91)	3.1±0.6	3.0±0.4
20-21	3.8±0.5	3.5*±0.5 (92)	3.7±0.5	3.5±0.5
4-21	39.7±4.2	38.5±3.1	38.6±3.9	37.8±3.1

[a] Data obtained from 111-121, MRID 46214402.
* Statistically significantly different from control, p≤ 0.05
** Statistically significantly different from control, p≤ 0.01.
Number in parentheses is % of control value, calculated by reviewer.
N=31-34

Body weight was measured in male and female pups in subsets III and IV (weeks 0-5 post-weaning), V (weeks 0-1 post-weaning) and VI (weeks 0-6 post-weaning). In subset III, body weight of treated females was significantly increased throughout post-weaning. Body weight gain in treated males was significantly decreased in weeks 2-3 but overall body weight gain was comparable to controls. Body weight gain in treated females was significantly increased during weeks 0-1 and 3-4. There were no significant changes in any other subsets. The findings are not considered toxicologically significant.

3. Developmental landmarks:

a. Sexual maturation: Preputial separation in males occurred between PNDs 40 and 47; the mean age was 43.1 and 43.5 for the control and treated groups, respectively. Vaginal opening occurred between PNDs 29 and 36; the mean age was 31.6 and 31.3 days for the control and treated groups, respectively. Body weight at attainment of sexual maturation was similar between the treated and control groups for males and females. The data are presented in Table 22.

Table 22. Mean (±SD) age and body weight at sexual maturation [a]		
Parameter	Dose (mg/kg bw/day)	
	0	0.2
N (M/F)	30/30	30/30
Preputial separation		
Age (days)	43.1±1.3	43.5±1.4
Body weight (g)	186.4±15.65	186.3±8.64
Vaginal opening		
Age (days)	31.6±1.6	31.3±1.9
Body weight (g)	95.6±8.25	96.4±12.02

[a] Data obtained from pages 122-123, MRID 46214402.

4. Behavioral assessment:

a. Functional observational battery (subset IV): There were no treatment-related FOB findings on PNDs 4, 11, 21, 35, 45 or 60.

b. Motor/locomotor activity (subset IV): Total and interval activity data are presented in Table 23. The total distance moved was significantly increased for two intervals (8 and 9) as was the accumulated distance in treated males on PND 13. Some increases and decreases in individual intervals were observed in treated females on PND 13 and 17 and in treated males on PND 21. The total distance was non-significantly increased in treated females on PND 21.

Some significant increases in number of rearings were noted during single intervals on PND 13 in treated males and on PNDs 17 and 60 in treated females. The accumulated number of rearings was unaffected by treatment. The data are presented in Table 24. Habituation was apparent in all groups by PND 21 and both motor and locomotor activities increased as the animals aged.

TABLE 23. Interval and total locomotor activity (mean ±SD) [a]

Sub-session		Dose (mg/kg bw/day)			
		Males		Females	
		0	0.2	0	0.2
PND 13	1	455.6±88.2	430.5±73.3	445.4±155.8	586.9±178.0
	2	165.2±120.9	240.0±131.3	243.8±172.3	315.6±217.1
	3	270.0±254.6	145.7±167.3	234.3±211.4	264.2±254.0
	4	227.8±231.1	248.5±252.7	181.4±156.7	151.7±179.9
	5	112.3±100.9	260.5±286.7	90.3±101.0	347.6**±332.0
	6	119.9±145.2	293.6±358.4	121.8±154.9	241.8±279.1
	7	46.5±38.0	303.1±459.6	107.7±108.2	86.1±69.0
	8	61.5±32.2	328.4*±323.8	159.6±232.4	96.6±134.5
	9	51.4±24.1	342.8*±388.3	82.4±83.4	31.4±19.7
	10	60.4±53.6	127.5±165.1	63.4±42.4	58.1±30.2
	11	56.7±34.6	109.8±146.6	150.4±211.4	49.0±48.3
	12	50.2±39.8	225.8±373.9	178.7±297.6	42.9±33.1
	Sum 1-12	1677.5±578.3	3056.0*±1269.4	2059.2±607.2	2271.8±1100.7
PND 17	1	870.4±115.5	956.3±252.9	823.7±162.2	855.8±291.2
	2	650.6±261.6	630.1±347.6	520.3±167.2	600.0±296.2
	3	516.5±359.5	707.4±453.9	410.2±327.5	341.5±265.2
	4	338.1±351.7	349.6±396.3	252.3±426.6	208.3±197.7
	5	237.7±423.9	268.2±302.4	144.7±213.4	228.3±305.8
	6	248.2±411.1	266.5±443.0	54.6±88.9	184.4±269.9
	7	148.4±374.9	178.5±297.8	71.9±166.7	46.5±29.0
	8	247.4±540.6	168.7±281.3	46.0±70.9	167.4*±240.3
	9	234.3±495.7	106.4±268.7	19.8±17.6	193.0*±258.6
	10	213.4±524.0	84.1±133.5	18.1±14.2	167.5*±299.5
	11	265.3±545.2	32.5±28.7	49.6±75.6	192.6±204.2
	12	250.2±499.3	161.1±305.4	93.4±196.9	525.1*±399.9
	Sum1-12	4220.6±4250.3	3909.5±1436.0	2504.7±922.0	3710.5±1871.2
PND 21	1	1341.3±193.1	1267.4±220.0	1448.3±242.3	1365.8±236.0
	2	649.1±192.4	546.0±199.6	603.4±248.9	756.0±136.7
	3	396.4±190.0	505.4±194.7	400.6±238.1	440.1±187.5
	4	569.9±237.6	303.9**±176.2	241.0±231.3	308.9±192.6
	5	277.4±239.8	298.7±304.0	372.3±211.7	314.8±310.7
	6	138.5±199.6	249.9±301.5	262.8±283.6	193.3±323.1
	7	250.2±311.7	221.1±228.2	81.9±127.1	166.9±217.3
	8	252.4±317.8	236.2±284.0	27.2±29.0	148.7±273.3
	9	158.6±239.4	91.2±147.4	73.1±105.2	108.1±181.4
	10	131.9±243.3	158.3±201.8	194.9±258.9	112.0±272.8
	11	98.3±174.6	163.0±259.4	96.7±120.0	102.9±271.5
	12	179.0±303.2	33.2±26.4	140.8±204.4	97.0±171.9
	Sum 1-12	4442.8±2126.4	4074.1±1638.6	3942.6±1268.1	4114.5±2030.1
PND 60	1	1215.3±227.4	1290.0±133.0	1528.1±157.1	1510.1±285.0
	2	1006.4±169.1	1085.5±233.7	1169.4±197.3	1253.3±158.8
	3	904.1±213.2	860.8±154.4	1028.9±163.0	1066.0±179.2
	4	748.4±157.3	750.5±227.0	827.7±208.0	955.6±186.2
	5	689.8±188.1	677.0±264.6	924.4±283.8	792.9±234.9
	6	668.9±212.0	634.8±199.8	666.8±253.1	803.1±163.4

TABLE 23. Interval and total locomotor activity (mean ±SD) [a]

Sub-session		Dose (mg/kg bw/day)			
		Males		Females	
		0	0.2	0	0.2
	7	586.8±166.3	562.2±221.6	739.9±207.7	760.7±291.6
	8	572.6±198.8	651.6±205.7	630.2±357.9	561.9±310.2
	9	512.2±239.5	507.6±217.4	560.9±248.9	498.7±335.6
	10	510.6±262.9	424.2±293.2	539.5±315.8	421.3±245.7
	11	423.8±218.4	430.3±345.4	461.8±370.3	429.7±371.4
	12	490.8±289.0	249.7±275.0	274.1±315.3	328.0±255.9
	Sum 1-12	8329.5±1573.3	8124.3±2017.4	9351.7±1783.9	9381.4±954.3

[a] Data obtained from pages 220-235, MRID 46214402
N =10
* Statistically different from control, $p<0.05$

TABLE 24. Motor activity (rearing) - intervals and total (mean ±SD) [a]

Sub-session		Dose (mg/kg bw/day)			
		Males		Females	
		0	0.2	0	0.2
PND 13	1	8.5±9.1	8.4±6.3	9.4±6.9	9.7±11.1
	2	1.3±1.9	4.7±8.0	5.1±6.7	7.4±9.5
	3	4.8±7.1	2.1±3.6	4.0±6.6	5.3±8.7
	4	3.0±4.7	2.9±5.8	3.3±5.9	3.6±6.1
	5	1.6±3.2	1.7±3.3	3.2±6.3	7.5±12.3
	6	1.7±4.4	1.1±2.0	2.7±4.1	6.3±8.6
	7	0.7±1.6	2.4±3.8	1.3±2.6	0.6±.1.1
	8	0.2±0.6	3.5±7.4	2.0±4.7	0.9±2.8
	9	0.0±0.0	3.1*±4.1	0.7±1.9	0.1±0.3
	10	0.2±0.6	0.4±0.5	0.0±0.0	0.3±0.7
	11	0.0±0.0	0.9±1.7	1.0±1.9	0.0±0.0
	12	0.9±2.2	1.0±2.5	2.8±7.0	0.0±0.0
	Sum 1-12	22.9±29.3	32.2±35.9	35.5±27.3	41.7±45.5
PND 17	1	35.4±8.8	33.0±7.3	34.1±7.6	31.7±7.0
	2	32.9±13.0	23.3±12.5	24.8±8.6	24.3±10.7
	3	17.4±11.1	23.2±16.2	12.7±10.4	13.2±10.5
	4	15.1±13.8	15.0±17.9	10.2±14.2	6.2±8.1
	5	8.1±13.5	6.2±6.9	5.5±10.4	5.6±8.4
	6	6.8±11.8	5.4±9.8	1.1±3.1	3.6±6.1
	7	5.2±13.5	6.4±13.5	1.5±4.7	1.0±1.5
	8	5.3±11.4	3.1±7.2	0.9±1.9	4.5±8.7
	9	5.3±11.2	4.1±11.6	0.0±0.0	7.3±12.4
	10	3.7±11.0	2.2±5.9	0.0±0.0	4.8±11.0
	11	8.1±17.0	0.0±0.0	0.4±1.3	6.0±9.0
	12	8.2±18.0	3.6±8.3	2.7±8.5	14.1*±15.2
	Sum 1-12	151.5±110.6	125.5±56.1	93.9±34.9	122.3±68.2
PND 21	1	42.6±8.0	48.8±15.0	53.0±8.1	46.5±10.2
	2	26.8±10.3	30.0±8.3	30.6±11.8	32.1±7.0
	3	18.9±7.3	20.0±11.7	21.2±12.6	20.3±9.5
	4	20.9±13.3	12.4±9.1	10.3±11.3	10.6±7.3
	5	8.6±8.2	10.1±10.5	15.0±9.7	9.2±10.5
	6	5.6±8.8	6.6±8.8	9.2±11.2	6.9±11.7
	7	8.6±10.9	7.0±10.4	4.1±7.5	6.0±8.8
	8	9.2±12.7	7.6±9.8	0.2±0.6	4.2±7.2
	9	6.7±10.6	3.3±5.8	2.3±4.8	1.8±3.6
	10	3.6±7.1	5.3±8.3	7.5±10.2	2.2±6.6
	11	2.3±5.0	4.6±7.4	5.0±7.0	2.7±8.5
	12	3.9±7.0	0.6±1.3	4.9±8.6	1.6±2.8
	Sum 1-12	157.7±63.9	156.3±65.2	163.3±46.0	144.1±60.6
PND 60	1	35.5±7.4	34.6±4.9	45.0±7.2	42.8±7.6
	2	27.1±6.0	29.1±7.7	31.8±5.4	36.7±6.8
	3	22.9±8.8	25.6±7.7	31.5±5.3	30.3±9.7
	4	21.6±8.2	20.8±8.4	21.2±4.0	27.2*±6.7
	5	18.1±6.1	17.2±7.6	19.9±7.3	22.0±8.1

TABLE 24. Motor activity (rearing) - intervals and total (mean ±SD) [a]					
Sub-session		Dose (mg/kg bw/day)			
		Males		Females	
		0	0.2	0	0.2
	6	18.1±8.4	13.4±6.8	16.9±6.8	18.4±8.3
	7	12.6±6.5	12.6±4.2	17.2±4.9	18.9±8.0
	8	12.6±4.0	12.2±5.1	14.2±8.8	12.9±7.5
	9	10.8±6.4	8.6±5.9	11.7±6.0	11.6±9.0
	10	11.3±7.0	7.8±6.7	12.5±10.1	10.5±7.5
	11	8.2±5.7	8.7±7.3	10.7±10.8	9.2±8.2
	12	8.7±5.2	4.2±5.4	2.9±4.7	5.5±6.1
	Sum 1-12	207.5±38.8	194.8±54.7	235.5±36.7	246.0±32.5

[a] Data obtained from pages 236-251 in the study report, MRID 46214402
N =10
* Statistically different from control, p<0.05

c. **Auditory startle reflex habituation (subset III) :** The amplitude and latency data are presented in Tables 25 and 26, respectively. There was no treatment-related effect on overall maximum amplitude or latency. On PND 24, treated females had a significant decrease in latency but in only one trial. Habituation was apparent on both testing days in both sexes.

TABLE 25. Auditory startle amplitude (v) (mean ±SD) [a]					
	Trial Block	Dose (mg/kg bw/day)			
		Males		Females	
		0	0.2	0	0.2
PND 24	1	405.0±166.8	462.2±244.1	380.5±141.1	387.6±91.2
	2	434.3±199.2	502.6±259.6	414.5±148.7	361.8±96.8
	3	347.9±121.2	423.2±196.0	360.0±134.7	366.0±115.3
	4	388.7±133.8	485.6±216.5	356.2±92.5	306.8±69.3
	5	394.9±110.8	430.0±189.4	340.9±85.2	337.6±93.0
	Mean	394.1±134.3	460.7±194.3	370.4±103.8	352.0±63.6
PND 60	1	1322.3±991.8	1028.8±252.3	726.8±374.7	752.1±439.1
	2	972.3±1047.7	822.1±330.2	720.0±823.6	513.0±280.8
	3	984.0±1122.1	693.1±404.5	427.4±239.2	445.5±229.0
	4	659.4±655.8	531.8±296.0	395.0±190.6	428.1±143.6
	5	662.2±700.7	483.3±216.0	467.9±475.7	381.6±244.4
	Mean	920.0±882.5	711.8±200.9	547.4±405.4	504.1±218.4

[a] Data obtained from 252-255, MRID 46214402.
N=10

TABLE 26. Auditory startle: time to peak amplitude (mean msec ±S.D.) [a]

	Trial Block	Dose (mg/kg bw/day)			
		Males		Females	
		0	0.2	0	0.2
PND 24	1	33.1±4.7	36.4±13.3	34.2±7.8	32.6±8.2
	2	34.1±9.6	30.5±7.0	32.4±5.8	29.9±5.5
	3	31.0±4.7	27.8±5.0	30.0±7.4	25.2*±2.3
	4	29.6±5.2	29.4±5.8	26.9±5.4	25.9±3.2
	5	27.4±5.1	29.7±4.3	27.6±4.8	28.9±6.1
	Mean	31.1±4.0	30.8±4.3	30.2±4.2	28.5±3.9
PND 60	1	42.3±7.8	42.0±4.1	37.7±12.5	36.5±7.4
	2	33.0±8.3	39.2±7.6	34.8±13.9	31.1±7.4
	3	32.6±8.8	33.9±8.1	29.1±3.8	30.7±9.9
	4	29.9±6.2	29.0±5.7	27.4±5.1	28.6±5.2
	5	29.3±7.4	31.0±4.6	33.4±9.1	27.3±5.6
	Mean	33.4±6.1	35.0±3.1	32.5±7.2	30.8±5.4

[a] Data obtained from 256-259, MRID 46214402.
N=10
* Statistically different from control, $p<0.05$

d. <u>Learning and memory testing (subsets V and VI)</u>: Learning was not apparent during testing sessions as an increase in the number of animals with successful trials and a decrease in the time to completion over successive trials. The data are presented in Tables 27 (PND 23 and 30) and 28 (PND 60 and 67). No confidence could be placed in the control data.

TABLE 27. Water maze performance in PND 23 and 30 offspring [a]

Session/parameter		Dose (mg/kg bw/day)			
		Males		Females	
		0	0.2	0	0.2
Number (%) Animals Reaching Criteria					
Learning 1 (PND 23)	Trial 2	2 (20)	3 (30)	5 (50)	5 (50)
	Trial 3	9 (90)	8 (80)	5 (50)	8 (80)
	Trial 4	9 (90)	10 (100)	8 (80)	10 (100)
	Trial 5	10 (100)	9 (90)	10 (100)	9 (90)
	Trial 6	10 (100)	10 (100)	9 (90)	10 (100)
Memory (PND 30)		7 (70)	9 (90)	10 (100)	10 (100)
Learning 2 (PND 30)	Trial 2	1 (10)	1 (10)	0 (0)	1 (10)
	Trial 3	2 (20)	1 (10)	3 (30)	2 (20)
	Trial 4	1 (10)	4 (40)	2 (20)	3 (30)
	Trial 5	2 (20)	5 (50)	3 (30)	4 (40)
	Trial 6	2 (20)	3 (30)	4 (40)	6 (60)
Time (sec ± SD)					
Learning 1 (PND 23)	Trial 2	32±22.5	27±17.8	17±8.4	27±22.5
	Trial 3	12±5.9	15±9.2	38±42.4	14*±12.8
	Trial 4	9±3.0	10±4.7	17±11.1	9±6.6
	Trial 5	6±1.3	10±6.9	8±3.3	7±2.6
	Trial 6	4±1.2	7*±3.9	7±4.6	7±3.6
Memory (PND 30)		6±5.2	6±3.7	5±1.3	5±1.5
Learning 2 (PND 30)	Trial 2	16±7.1	15±5.0	12±3.6	19*±8.7
	Trial 3	14±7.6	11±4.4	13±4.2	15±10.0
	Trial 4	14±6.1	10±5.3	16±13.5	9±3.4
	Trial 5	11±4.5	9±5.0	13±7.0	9±6.6
	Trial 6	14±9.6	9±4.8	10±3.8	8±2.9

[a] Data obtained from pages 148-151, MRID 46214402.
* Statistically different from control, $p<0.05$
N=10

TABLE 28. Water maze performance in PND 60 and 67 offspring [a]

Session/parameter		Dose (mg/kg bw/day)			
		Males		Females	
		0	0.2	0	0.2
Number (%) Animals Reaching Criteria					
Learning 1 (PND 60)	Trial 2	6 (60)	4 (40)	4 (40)	3 (30)
	Trial 3	8 (80)	7 (70)	7 (70)	5 (50)
	Trial 4	8 (80)	5 (50)	4 (40)	7 (70)
	Trial 5	8 (80)	8 (80)	7 (70)	9 (90)
	Trial 6	9 (90)	9 (90)	6 (60)	9 (90)
Memory (PND 67)		10 (100)	8 (80)	6 (60)	7 (70)
Learning 2 (PND 67)	Trial 2	1 (10)	3 (30)	2 (20)	3 (30)
	Trial 3	4 (40)	3 (30)	3 (30)	3 (30)
	Trial 4	4 (40)	6 (60)	3 (30)	4 (40)
	Trial 5	5 (50)	7 (70)	5 (50)	6 (60)
	Trial 6	7 (70)	7 (70)	5 (50)	3 (30)
Time (sec ± SD)					
Learning 1 (PND 60)	Trial 2	18±10.0	22±16.2	17±8.8	16±5.9
	Trial 3	13±14.9	10±7.6	13±10.1	10±4.5
	Trial 4	8±5.1	10±6.5	21±22.6	13±16.8
	Trial 5	9±12.1	7±4.3	9±6.4	6±3.5
	Trial 6	5±3.3	7±7.6	16±16.5	7±6.3
Memory (PND 67)		6±4.1	9±7.3	7±4.7	8±6.0
Learning 2 (PND 67)	Trial 2	23±15.3	22±20.1	15±6.3	16±13.4
	Trial 3	17±11.5	17±12.0	11±6.6	12±7.1
	Trial 4	13±8.2	9±6.0	21±18.6	11±11.2
	Trial 5	8±3.7	7±3.6	19±22.7	11±13.8
	Trial 6	6±3.2	7±5.1	11±11.6	15±10.9

[a] Data obtained from pages 152-155, MRID 46214402.
N=10

5. **Cholinesterase activity:** Results of cholinesterase (ChE) activity assessment are presented in Table 29. There were no treatment-related significant ChE changes in male and female offspring on PND 4.

   On PND 21, significant inhibition in serum (21%) and RBC (13%) enzyme activities were observed in treated dams; brain ChE was non-significantly inhibited by 18%. On PND 21, significant inhibition was observed for serum (39-49%), RBC (47-56%), and brain (38-48%) enzyme activities in treated male and female offspring.

TABLE 29. Cholinesterase activity in dams and offspring

Cholinesterase (mean ± SD)	Dose (mg/kg bw/day)	
	0	0.2
Lactation day 21 dams		
Serum (µkat/L)	19.7±4.3	15.6**±2.0 (21)
RBC (µkat/L)	37.8±2.8	33.0**±2.9 (13)
Brain (µkat/g)	2.8±1.2	2.3±1.0
Day 4 male offspring		
Serum (µkat/L)	13.0±1.5	13.1±0.4
RBC (µkat/L)	15.6±3.8	15.2±3.7
Brain (µkat/g)	1.2±0.1	1.2±0.1
Day 4 female offspring		
Serum (µkat/L)	13.2±1.1	13.1±1.5
RBC (µkat/L)	16.0±5.1	15.8±1.7
Brain (µkat/g)	1.3±0.1	1.2±0.0
Day 21 male offspring		
Serum (µkat/L)	14.3±1.5	7.3**±1.1 (49)
RBC (µkat/L)	41.4±5.7	18.4**±3.3 (56)
Brain (µkat/g)	2.9±0.7	1.5**±0.2 (48)
Day 21 female offspring		
Serum (µkat/L)	13.0±2.2	7.9**±1.1 (39)
RBC (µkat/L)	38.3±7.0	20.3**±3.4 (47)
Brain (µkat/g)	2.6±0.5	1.6**±0.3 (38)

Data obtained from pages 284-288, MRID 46214402.
N=10
**Statistically different from control, $p<0.01$
Number in parantheses is % inhibition relative to control value calculated by reviewer

6. Postmortem results (subsets II and III):

a. **Brain weight:** On PND 22, terminal body weight was significantly decreased (94% of control value) in treated males. On PND 60 (±2), the absolute brain weight was significantly increased (104% of control value) in treated females. Mean brain weight data are presented in Table 30.

TABLE 30. Mean (±SD) brain weight data in offspring [a]				
Parameter	Dose (mg/kg bw/day)			
	Males		Females	
	0	0.2	0	0.2
Day 22				
Terminal body weight (g)	52.73±5.06	49.54*±3.25	50.37±6.29	50.44±3.82
Brain weight (g)	1.65±0.05	1.67±0.04	1.63±0.11	1.57±0.08
Brain-to-body weight ratio (%)	3.17±0.36	3.38±0.27	3.25±0.26	3.11±0.14
Day 60±2				
Terminal body weight (g)	286.17±29.15	269.26±18.72	176.39±7.26	187.41±12.10
Brain weight (g)	2.03±0.09	1.95±0.08	1.83±0.05	1.90*±0.06
Brain-to-body weight ratio (%)	0.71±0.06	0.73±0.03	1.04±0.04	1.02±0.07

[a] Data obtained from pages 289-296, MRID 46214402
N=10
* Statistically significantly different from control value, $p<0.05$

b. Macroscopic examination: No treatment-related effects were reported for male or female offspring on PND 22 or 60±2.

c. Neuropathology (subsets II and III):

1) **Microscopic examination:** No treatment-related effects were reported for male or female offspring on PND 22 or 60 (±2). On PND 60 (±2), axonal degeneration was observed in two control males in the proximal sciatic nerve, in one control male in the distal tibial nerve and in the proximal nerve of one treated male and one female. One treated female had focal calcification at the region of the meninges of the forebrain and cortex. These findings are not considered treatment-related.

2) **Brain Morphometry:** Morphometric evaluation, presented in Table 31 revealed the following: significant decrease in the cerebrum length and width in treated males on PND 60±2; significant decrease in neocortex (FC left) and neocortex (PC left) in treated males on PND 22; significant decrease in neocortex (FC right) and cerebellum (Folpyr half) in treated females on PND 22.

TABLE 31. Mean (±SD) brain morphometric data [a]				
Parameter	Dose (mg/kg bw/day)			
	Males		Females	
	0	0.2	0	0.2
Day 22				
Cerebrum				
Length (cm)	1.38±0.02	1.39±0.03	1.39±0.04	1.37±0.02
Width (cm)	1.47±0.01	1.45±0.03	1.44±0.03	1.43±0.04
Cerebellum				
Length (cm)	0.71±0.02	0.71±0.02	0.71±0.04	0.69±0.04
Width (cm)	1.10±0.02	1.10±0.02	1.10±0.03	1.10±0.02
FC Left (μm)	1568±54	1454**±105 (93)	1518±113	1501±95
FC Right (μm)	1553±57	1490±133	1518±108	1434*±99 (94)
PC Left (μm)	1568±35	1503*±90 (96)	1526±105	1537±81
PC Right (μm)	1573±28	1520±147	1500±101	1504±66
Folpyr half (μm)	335±35	326±35	311±19	282**±17 (91)
Termination				
Cerebrum				
Length (cm)	1.53±0.02	1.48**±0.03 (97)	1.44±0.02	1.46±0.04
Width (cm)	1.53±0.03	1.50**±0.03 (98)	1.47±0.03	1.48±0.03
Cerebellum				
Length (cm)	0.75±0.01	0.74±0.04	0.75±0.03	0.74±0.03
Width (cm)	1.17±0.04	1.15±0.03	1.14±0.03	1.15±0.02

[a] Data obtained from pages 301-308, MRID 46214402.
FC Left = frontal cortex, left brain; FC right = frontal cortex, right brain; PC Left = parietal cortex, left brain; Folpyr half = folium pyramis of cerebellum, half way between tip and base
N = 10
* Statistically significantly different from control value, $p<0.05$
** Statistically significantly different from control value, $p<0.01$

III. DISCUSSION and CONCLUSIONS (MRIDs 46214401 and 46214402):

A. INVESTIGATORS' CONCLUSIONS:

MRID 46214401: The high dose of 0.3/0.2 mg/kg/day evoked dam mortality and marked maternal systemic toxicity which lead to a high number of stillborn pups and excessive pup mortality in early lactation. The high dose was terminated after approximately the first week of lactation. The study author concluded that the NOAEL for developmental neurotoxicity was 0.1 mg/kg/day. A supplementary developmental neurotoxicity study was initiated where a dose of 0.2 mg/kg/day was compared to a control group.

MRID 46214402: The study author concluded that the NOAEL for developmental neurotoxicity was 0.2 mg/kg/day.

B. REVIEWER COMMENTS: Two concurrent studies evaluated the developmental neurotoxicity of Phorate in rats. No treatment-related clinical signs of toxicity or effects on body weight and food consumption were observed in any of the other treated groups, up to and including 0.2 mg/kg bw/day. The high dose of 0.3 mg/kg bw/day was excessively toxic to both maternal animals and offspring. At this dose, six dams died during gestation and lactation and clinical signs of toxicity were observed in both premature decedents and survivors. Clinical signs of toxicity in high-dose dams included tremors, high stepping gait, poor general state, labored respiration, salivation after treatment and chromodacryorrhea. Body weight during gestation and lactation was only slightly affected in dams administered 0.3 mg/kg bw/day. In addition, body weight gain for the high-dose group was decreased during LDs 1-2. Body weight loss for this group was accompanied by decreased food consumption during early lactation. No biologically significant inhibition of serum, erythrocyte (RBC) or brain ChE activities was found in dams on lactation day 21. However, there was a modest decrease in RBC cholinesterase. Statistical significance attained for serum and RBC inhibition is considered to be an artifact of the analysis that was used.

No treatment-related effect on the mean number of pups delivered per dam was seen. However for females at 0.3 mg/kg bw/day, the number of live born pups was decreased and the number of stillborn pups was increased compared with the control group. Correspondingly, the live birth index was decreased for this treated group. Pup growth and viability were markedly decreased at 0.3 mg/kg bw/day during LDs 1-4 probably as a consequence of maternal neglect. Although the high dose was reduced at the end of gestation/early lactation, maternal mortality and small litter size due to increased pup mortality necessitated termination of this group before direct dosing of offspring commenced. Offspring survival in the other treated groups was similar to the controls and no clinical signs of toxicity were observed.

In the 0.3 mg/kg bw/day group, male and female offspring body weights were lower than those of the controls on PNDs 1 and 4 and body weight gains were decreased during this interval. A trend for decreased weight gain by males in the 0.1 and 0.2 mg/kg bw/day groups is suggested during late lactation. However, the magnitude of the differences in the mean values between the treated and control groups was less than the standard deviation about the mean for the treated group at each interval that attained statistical significance. In addition, absolute body weight was not affected at any time during lactation, apparent differences in weight gain were found only for overnight intervals, and statistical analysis may not have been appropriate. Therefore, decreased body weight gain by male pups at 0.1 and 0.2 mg/kg bw/day is not considered treatment-related or biologically significant in the current study.

During the post-weaning interval, body weight and body weight data were considered separately for each subset. Although statistical significance was occasionally found for body weight or body weight gain during the post-weaning interval, no dose- or treatment-related trends were observed. The mean day of sexual maturation and body weight at attainment were not affected by treatment.

No treatment-related effects were seen on clinical signs, FOB parameters, developmental landmarks, brain weights, brain morphology or neuropathology.

No conclusions can be made with regard to the effect of phorate on offspring learning and memory due to conflicting results between the two studies. When the same testing methods used, learning was apparent in the main study but not in the supplemental study. Consequently, no confidence can be placed in learning and memory assessment.

A treatment-related increase in the motor activity was seen in both sexes of pups on PND 21 at the at 0.1 mg/kg/day. The increase, over controls, were 38% in males and 21% in females. An increase is also noted at the low dose (0.03 m/k/d) in males (9%) and females (10%); however, these increases were determined to be not toxicologically significant due to the lesser magnitude of the effect.

A clear treatment-related decreases in mean peak amplitude response was seen in the males on PND 60 at 0.1 mg/kg/day. This decrease is supported by a smaller magnitude of decrease in males on PND 24 and by habituation data. The effect is consistent across blocks. Data from the follow-up study at 0.2 mg/kg/day support the results seen at 0.1 mg/kg/day.

No treatment-related inhibition of serum, RBC, or brain ChE activity was found in either sex on PND 4. At 0.1 mg/kg/day significant inhibition was seen in plasma (15%), RBC (16%) and brain (21%) in males on Day 21; only plasma (19%) was inhibited in females on that day. At 0.2 mg/kg/day, significant inhibitions were seen in all three compartments.

Based on the combined results of both studies, the maternal systemic LOAEL is 0.3 mg/kg /day based on death, clinical signs of toxicity, decreases in body weight gain during gestation and reduced food consumption during lactation. The maternal NOAEL is 0.2 mg/kg bw/day.

The maternal cholinesterase NOAEL is 0.2 mg/kg/day; a LOAEL is not established.

The offspring systemic and cholinesterase inhibition LOAEL is 0.1 mg/kg bw/day based on increases in motor activity in both sexes on PND 21, decrease in auditory startle reflex in males on PND 60 and plasma, RBC and brain cholinesterase inhibition in males on Day 21. The offspring NOAEL is 0.03 mg/kg bw/day.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessment of learning and memory in the offspring and the pending review of the of positive control data.

C. STUDY DEFICIENCIES:.

The learning memory assessment used the same testing methods. However, learning was demonstrated in the main study but was not demonstrated in the supplemental study. The registrant should provide rationale for this discrepancy.

13544

R115883

Chemical: **Phorate**

PC Code:
057201
HED File Code: **13000 Tox Reviews**
Memo Date: **9/21/2005**
File ID: **TX0052433**
Accession #: **412-06-0011**

HED Records Reference Center
9/14/2007