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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TXR#: 0053278

DATE: October 4, 2005

SUBJECT: Dicrotophos: Developmental Neurotoxicity Studies Review

FROM: Santhini Ramasamy, PhD, DABT, MPH Santhini Ramasamy 10-5-05
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TASK ID: DP Code: D316124
P.C. Code: 035201

REGISTRANT: AMVAC Chemical Corporation, Los Angeles, CA

I. Action Requested

Complete the review of the developmental neurotoxicity study (MRID 46153201 and MRID 46153202) for dicrotophos.

II. Conclusions

Attached is the Data Evaluation Record of the developmental neurotoxicity study. The DNT Workgroup of HED concluded that the main developmental neurotoxicty 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 lack of brain morphometric data at the low and mid dose groups and pending review of the positive control data.

EPA Reviewer: Santhini Ramasamy, PhD, DABT, MPH
Reregistration Branch 4, Health Effects Division (7509C)

Signature Santhini Ramasamy
Date 10-5-05

EPA Secondary Reviewer: William Dykstra Ph.D.
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Signature: W. Dykstra
Date 10-5-05

TXR#: 0053278

DATA EVALUATION RECORD

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

PC CODE: 035201

DP BARCODE: D316124
SUBMISSION NO.: SXXXXXX

TEST MATERIAL (PURITY): Technical Grade Dicrotophos (87.6%)

SYNONYMS: Bidrin

CITATION: Brammer, A. (2003) Dicrotophos: Developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK104TJ. Study Number RR0884; October 24, 2003. MRID 46153202. Unpublished.

Milburn, G.M. (2003). Dicrotophos: Preliminary Developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderly Park, Macclesfield, Cheshire, UK SK104TJ. Study ID RR0883. October 14, 2003. MRID 46153201. Unpublished.

SPONSOR: AMVAC Chemical Corporation, 4100 East Washington Blvd,, Los Angeles, California, CA 90023.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46153202), dicrotophos (87.6% a.i., batch # 403001B) was administered by gavage to 30 parent female Alpk:AP$_f$SD Wistar-derived rats/dose by gavage at dose levels of 0, 0.01, 0.05 or 0.4 mg/kg/day from gestation day (GD) 7 through parturition to lactation day 7. Selected pups were dosed by gavage with 0, 0.01, 0.05 or 0.4 mg/kg/day from post natal days (PNDs) 8 to 22; pups were weaned on day 29 and the study was terminated on PND 63. Doses were selected on the basis of a range-finding study (MRID 46153201). A Functional Operational Battery (FOB) was performed on 30 dams/dose on GDs 10 and 17, and lactation days 2 and 9. On PND 5, litters were culled to yield four males and four females (as closely as possible). Offspring selected for F_1 generation were allocated (one/sex/litter) FOB, assessment of motor activity, assessment of auditory startle response habituation, assessment of learning and memory, and neuropathology at

study termination (day 63 of age). On days 12 and 63, the whole brain was collected from 10 pups/sex from control and high dose groups for micropathologic examination and morphometric analysis. Pup physical development was assessed by body weight, and sexual maturation of females was assessed by age at vaginal opening. Maturation of males was assessed by age at completion of preputial separation. Cholinesterase activity, either in the dams or the offspring, was not measured in this study.

In dams during gestation and lactation, no treatment-related effects on mortality, clinical signs, body weight, body weight gain, food consumption, FOB, or reproductive parameters were noted. **The maternal systemic NOAEL is ≥0.4 mg/kg/day, the highest dose tested. The maternal LOAEL is not identified.**

In offspring, there were no treatment-related deaths or clinical signs or effects on birth weight, body weight or body weight gain pre- or post-weaning, clinical observations, developmental landmarks, FOB parameters, motor or locomotor activity, auditory startle response, or learning and memory tests. At necropsy, no treatment-related gross lesions or changes in absolute weight were seen.

The brain morphometric analyses revealed statistically significant decreases (9-14%↓) in cortex measurements in males at PND 12 and the overall width of the thalamus/cortex (5%↓) in males at PND 63 . In females, measurements of the hippocampus at various levels are significantly increased (7-10% ↑) at PND 12 and the hippocampus length and thalamus width are decreased (4-11%↓) at PND 63. These changes are considered related to treatment. Because of the changes seen at the high dose, morphometric data for all tissues (cortex, hippocampus, and thalamus) for the low and mid dose groups should be evaluated.

The offspring systemic LOAEL is 0.4 mg/kg/day based on morphometric changes in the brain of male and female offspring on PND 12 . The NOAEL is not established.

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 lack of brain morphometric data at the low and mid dose groups and pending review of the positive control data.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Technical grade Dicrotophos

Description:	Clear brown liquid
Lot/Batch #:	403001B
Purity:	87.6 % a.i.
Compound Stability:	expiry date May 2003
CAS # of TGAI:	Not Available

2. **Vehicle and/or positive control:** Controls received deionized water. No positive control was maintained.

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

Species:	Rat	
Strain:	Alpk:AP$_f$SD Wistar-derived	
Age at study initiation:	Females: time-mated, 10-12 wks old	
Wt. at study initiation:	229.0-297.0 g	
Source:	Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire. U.K.	
Housing:	Individually in solid plastic cages	
Diet:	Powdered CTL diet, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±3°C
	Humidity:	30-70%
	Air changes:	at least 15/hour
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 6 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: October 11, 2002; End: July 30, 2003

2. **Study schedule:** The maternal animals were time mated at the testing laboratory's Rodent Breeding Unit and assigned to study. The test substance was administered to the maternal animals (30/dose group) from gestation day (GD) 7 through lactation day 7. Selected pups were dosed by gavage from post natal days (PNDs) 8 to 22. Pups were weaned on PND 29, after which maternal animals were killed. Pups selected for F_1 generation remained on study up to PND 63.

3. **Mating procedure:** Females were paired with males (ratio of female to male not specified) of the same strain and source. Each female was examined during the mating period to identify sperm cells in a vaginal smear. The day that sperm was found was designated gestation day 1. After successful mating, each pregnant female was placed individually in a solid plastic cage with bedding, where the dam was maintained through gestation and post partum period.

4. **Animal assignment:** Mated females and offspring were allocated as shown in Table 1 using a randomized block design. For offspring, four sets of animals (designated sets A-D) were utilized for assessment at each age. Randomly-selected pups (Set D; 10/sex/dose) were perfused with fixative and brains were collected for histopathological examination and morphometric analysis.

   One pup/sex/litter was allocated on PND 5 to each of the following: functional observational battery, motor activity, auditory startle habituation, learning and memory (water maze), as well as for sacrifice and brain examination on day 12 and 63. No opthalmoscopic examination of pups was conducted. On day 63, animals were sacrificed by perfusion and brain weight was recorded.

TABLE 1. Study design

Experimental parameter		Dose (mg/kg/day)			
		0	0.01	0.05	0.4
Maternal animals					
		No. of maternal animals assigned			
FOB (GD 10,17)		30	30	30	30
FOB (PND 2, 9)		30	30	30	30
Offspring					
Set A	Functional Observation battery (PND 5, 12, 22, 36, 46 and 61)	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Set B	Motor activity (PND 14, 18, 22 and 60)	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Set C	Auditory startle habituation (PND 23, 61)	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
	Water maze (PND 24, 27)	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
	Associative Learning and memory (PND 59, 62)	1/sex/litter	1/sex/litter	1/sex/litter	1/sex/litter
Sets A-C	Brain Weight (PND 12, 63)	10/sex	10/sex	10/sex	10/sex
Set D	Gross necropsy and brain measurements (PND 12, 63)	10/sex	- -	- -	10/sex

5. **Dose selection rationale:** Dose levels were chosen based on the results from a preliminary developmental neurotoxicity study (MRID 46153201; CTL Study No. RR0883) and a repeat dose cholinesterase inhibition study in pre-weaning and young adult pups (MRID 46153204/ CTL Study no,. KR1491). In a preliminary DNT study in rats (see Appendix), dicrotophos administered at dose levels of 0, 0.05, 0.2 and 1.0 mg/kg/day caused dose-related inhibition of erythrocyte and brain cholinesterase activity in dams. On GD 22 fetuses from the 0.2 and 1.0 mg/kg/day had inhibition of erythrocyte and brain cholinesterase activity. Inhibition of cholinesterase activity was noted in offspring on PND 2 but there was no evidence of inhibition from PND 8 onwards.

   The cholinesterase inhibition study (CTL Study No. RKR1491) was not available for review but was summarized in the report. In that study, dicrotophos was administered by gavage at

dose levels of 0, 0.008, 0.02, 0.08 or 0.4 mg/kg/day for 7 days to pups of 12 or 42 days of age. In both pre-weaning and young adult pups, the brain and erythrocyte cholinesterase activity was significantly inhibited at 0.4 mg/kg/day. The lowest dose shown to cause inhibition of erythrocyte cholinesterase activity in pre-weaning pups was 0.08 mg/kg/day. There were no effects at 0.008 or 0.02 mg/kg/day and no sex differences were seen.

Based on the results of these studies, the doses selected for the developmental neurotoxicity study were 0.01, 0.05 and 0.4 mg/kg/day. The 0.4 mg/kg/day dose level was selected because it was shown to cause inhibition of brain and erythrocyte cholinesterase activity on pre-weaning and young adult pups (CTL Study No. KR1491). The mid dose of 0.05 mg/kg/day was also shown to cause inhibition of erythrocyte and brain cholinesterase activity in dams (CTL Study No. RR0883). The 0.01 mg/kg/day was selected to be the NOEL since it was not expected to cause effects on cholinesterase activity.

6. **Dosage administration:** Dicrotophos (1ml/100g body weight) was administered daily to parent females by gavage at dose levels of 0, 0.01, 0.05 or 0.4 mg/kg/day from GD 7 through lactation day 7, inclusive. The selected offspring were dosed once daily by gavage from PND 8 to PND 22, inclusive. Dosing was performed sequentially, in group order, at approximately the same time each day.

7. **Dosage preparation and analysis:** The dose preparations were made at weekly intervals. A stock solution was prepared by adding deionized water to a weighed amount of test article and each dose was prepared by further dilution of the stock solution. The control group received deionized water. Dose preparations were subdivided into aliquots for daily dosing and stored at 4°C until required. The daily doses were kept at room temperature for 1 hour prior to dosing. Homogeneity analysis was not done because the preparations were considered to be solutions. The stability of dicrotophos in water was demonstrated in the earlier study (CTL Study No. KR1491). Concentrations of the test substance in the dose preparations were analyzed by gas chromatography on three occasions.

Results:

Homogeneity analysis: was not conducted.

Stability analysis: The stability of dicrotophos in water for up to 12 days when stored at +4 °C was demonstrated in an earlier study (CTL Study No. KR1491) at dose levels of 0.0008 mg/ml and 0.4 mg/ml was found to be stable; these data were not included.

Concentration analysis: The 0, 0.01, 0.05 or 0.4 mg/kg/day dose levels averaged 99%, 98.5%, and 100.4% of the nominal concentration, respectively. The mean concentrations of dicrotophos in deionized water were within 2% of the nominal concentrations.

The analytical data indicated that the concentration and stability of dicrotophos in deionized water were adequate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals**: Twice daily cage-side observations were conducted for maternal animals. Detailed clinical observations were recorded immediately prior to dosing on GDs 1 through lactation day 7, and on lactation days 15 and 22 and on the day of termination (day 29).

Thirty dams per group were observed (by observers blind to the treatment group) outside the home cage during the gestation dosing period (days 10 and 17) and at least 10 dams/group were observed during the post partum period (days 2 and 9). The functional observation battery included, but was not limited to, the following:

	Functional observations–Maternal animals
X	Signs of autonomic function, including: presence or absence of lacrimation and salivation, piloerection, exophthalamus, urine staining, diarrhea, ptosis, and pupillary response to light.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal motor movements, both in the home cage and standard (open) arena.
X	Ranking by severity of subject's reactivity to general stimuli.
X	Ranking by severity of subjects's arousal level or state of alertness during observations of the unperturbed subject in the standard (open) arena.
X	Description and incidence of posture and gait abnormalities, both in the home cage and standard (open) arena.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypes), emaciation, dehydration, changes in muscle tone, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

The individual maternal body weights were recorded upon arrival at CTL, and prior to dosing on GD 1 through lactation day 7, and on lactation days 15 and 22 and on the day of termination (day 29).

Females showing signs of difficult parturition, and those failing to litter by day 25 of gestation or those that failed to maintain their litters to day 29 post partum were sacrificed. The remaining females were permitted to deliver and rear offspring until postnatal day 22. Number of live and dead offspring were recorded during parturition.

b. **Offspring**:

1) **Litter observations**: Each litter was examined soon after completion of parturition (day1), and within 24 hours. Daily throughout lactation, litters were examined for dead or abnormal pups.

On day 5 postpartum, litters were standardized to 8 randomly selected pups/litter (4/sex/litter, as nearly as possible); the surplus litters were sacrificed after weighing without routine necropsy. Litters of 7 or 8 pups with at least 3 male and 3 female pups were used for the selection of the F1 generation.

2) **Body weight/Detailed observation:** From PND 5, the selected offspring were examined for clinical signs and body weight was determined immediately prior to dosing on PNDs 8-22, on PNDs 29, 36, 43, 50, 57 and 63 and prior to termination. The examinations were made by observers who were aware of the treatment groups.

3) **Developmental landmarks:** Beginning on PND 36, selected F_1 males were examined daily for preputial separation. Beginning on PND 29, selected F_1 females were examined daily for vaginal opening. The age of onset and body weight at attainment were recorded.

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

i) **Functional observational battery (FOB):** On PNDs 5, 12, 22, 36, 46, and 61, one male or one female from each litter was examined outside the home cage by observers blind to the treatment groups. On PNDs 5, 12 and 22, the examination was made prior to dosing. Otherwise, methods were similar to the procedures used for the dams.

FUNCTIONAL OBSERVATIONS- Offspring	
X	Signs of autonomic function, including: presence or absence of lacrimation and salivation, piloerection, exophthalamus, urine staining, diarrhea, ptosis, and pupillary response to light.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements, both in the home cage and standard (open) arena.
X	Ranking by severity of subject's reactivity to general stimuli.
X	Ranking by severity of subjects's arousal level or state of alertness during observations of the unperturbed subject in the standard (open) arena.
X	Description and incidence of posture and gait abnormalities, both in the home cage and standard (open) arena.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, changes in muscle tone, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

ii) **Motor activity testing:** Motor activity was evaluated in one male and one female from each litter on PNDs 14, 18, 22, and 60. On PNDs 14, 18 and 22, the assessment was made prior to dosing. The tests were conducted in a separate room to minimize disturbance and used an automated activity recording apparatus to record small and large movements as an activity count. Each assessment consisted of 10 scans of five minute duration. The treatment groups were counterbalanced across cage/device numbers (up to

32 animals/trial/run). For repeat trials each animal was returned to the same activity monitor. Habituation was evaluated as a decrease in activity over consecutive trial-session intervals.

iii) **Auditory startle:** The auditory startle habituation test was performed on one male and one female from each litter on PNDs 23 and 61, using an automated recording apparatus. The mean response amplitude and time to maximum amplitude in each block of 10 trials (5 blocks of 10 trials per session on each day of testing) were calculated. No further description of the testing procedure was given.

iv) **Learning and memory testing/Water Maze:** On PNDs 24 and 27, learning and short- and long-term memory retention testing was performed in one male and one female from each litter using a 'Y'- shaped water maze with one escape ladder. The time taken by the animal to find the escape ladder was recorded for each of 6 trials. A straight channel was also used to evaluate swimming speed. Each animal completed 1 trial in the straight channel immediately following the 6 trials in the 'Y'- shaped water maze. The percentage of successful trials in the Y-maze were calculated separately for each animal. The criterion for a successful trial was a time less than a specified cut-off time. Cut-off time values of 3, 4, 5, 6, 7, 8, 9, and 10 seconds and 1, 1.5 and 2 times the straight channel time were used. Alternative pups, one per litter selected on day 5 post partum were tested for associative learning and memory test on days 59 and 62 , respectively, and the procedure used for days 24 and 27 was repeated.

5) **Opthalomology:** No opthalmoscopy was performed on offspring.

2. **Postmortem observations:**

a. **Maternal animals:** Maternal animals were sacrificed by haloethane vapor inhalation followed by exsanguination on day 29 post partum and discarded without examination. Three females with difficult parturition and one showing clinical signs were sacrificed using the same procedure. They were subjected to a macroscopic examination to confirm the pregnancy status but no tissues were collected.

b. **Offspring:** The offspring found dead during the dosing phase were subjected to gross necropsy, otherwise animals found dead were discarded. Offspring not selected for F_1 generation on PND 5 post partum were sacrificed and discarded. The F_1 animals selected for brain weight or neuropathological evaluation were sacrificed on day 12 or 63. These animals were subjected to postmortem examination as described below.

On day 12, up to 10 pups/sex/group were sacrificed by exposure to carbon dioxide. The brain was collected and immersion fixed in 10% buffered formol saline. Approximately 24 hours after fixation, the brains were weighed. Only the brains from rats in the control and high dose groups were embedded in paraffin wax, sectioned into 7 levels at 5 μm and stained with hematoxylin and eosin. Levels 2-5 in the brain and the sections of cerebellum

were examined and selected for morphometric measurements using a KS400 image analysis system.

The following brain morphometric measurements were made:

Frontal cortex height and width

Dorsal cortex thickness (dorsal portion of the cerebral cortex within the coronal section)

Pyriform cortex thickness (at midpoint between rhinal and amygdaloid fissures, both sides)

Hippocampus length (from midline to outer edge of most lateral pyamidal cells, both sides)

Hippocampal dentate gyrus width and length (greatest dorsal-ventral thickness)

Thalamus height and width

Thalamus/cortex width

Corpus callosum (thickness at the midline)

Cerebellum (height and length)

Preculminate fissure (thickness of outer granular, molecular, and inner granular layers)

Prepyramidal fissure (thickness of outer granular, molecular, and inner granular layers)

On day 63, up to 10 rats/sex/group were sacrificed by exposure to carbon dioxide and the brains were removed and weighed prior to being fixed in formol saline. An additional 10 rats/sex/group were anesthetized by intraperitoneal injection of sodium pentobarbitone and killed by perfusion fixation with formol saline. Following perfusion fixation, brains were removed and weighed. The volume of fixative used was approximately equivalent to the estimated body weight. The following tissues were preserved in "appropriate fixative:" brain, eyes with optic nerves and retina, spinal cord including cervical and lumbar swellings, spinal nerve roots (both dorsal and ventral root fibers) and dorsal root ganglia at cervical and lumbar swellings, proximal sciatic and tibial nerves, distal tibial nerve (tibial nerve calf muscle branches) and gastocnemius muscle. Only the left side nerves were processed while the right side nerves were stored. Tissues from the control and high-dose animals were processed and examined by light microscopy. CNS tissues and skeletal muscle were embedded in paraffin wax, sectioned at 5 μm, and stained with hematoxylin and eosin. PNS tissues were embedded in resin and semi-thin sections were cut and stained with toluidine blue.

The following brain morphometric measurements were performed:

Frontal cortex height and width

Dorsal cortex thickness (dorsal portion of the cerebral cortex within the coronal section)

Pyriform cortex thickness (at midpoint between rhinal and amygdaloid fissures, both sides)

Hippocampus length (from midline to outer edge of most lateral pyamidal cells, both sides)

Hippocampal dentate gyrus width and length (greatest dorsal-ventral thickness)

Thalamus height and width

Thalamus/cortex width

Corpus callosum (thickness at the midline)

Cerebellum (height and length)

Preculminate fissure (thickness of outer granular, molecular, and inner granular layers)

Prepyramidal fissure (thickness of outer granular, molecular, and inner granular layers)

D. DATA ANALYSIS:

1. **Statistical analyses**:. Maternal and pup body weights, total litter weight, gestation length and litter size were analyzed by ANCOVA or ANOVA. The proportion of litters with gestation length ≤ or ≥22 days, the proportion of whole litter loss in each treated group, the proportion of pups born live, the proportion of pups surviving, the proportion of litters with all pups born live and with all pups surviving, and the proportion of male pups as well as the proportion of males and females with observed preputial separation or vaginal opening were analyzed by Fisher's Exact Test. The percentage of live born pups, pre-and post-cull pup survival and pup sex were analyzed by ANOVA following the double arcsine transformation of Freeman and Turkey (1950).

   Motor activity measurements and startle response maximum amplitude and time to maximum amplitude data, as well as the litter-based mean age and mean body weight at attainment of sexual maturation were analyzed by ANOVA, separately for males and females. Swimming times in the straight channel, each individual trial in the Y-maze as well as the percentage of successful trials in the Y-maze were analyzed by ANOVA following the double arcsine transformation of Freeman and Turkey (1950), separately for males and females. Brain weight and brain morphology data were analyzed by ANOVA and by ANCOVA on final

body weight, separately for males and females. All analyses were carried out in SAS (1999). The proportion in each treated group was compared to the control group proportion by Fisher's Exact Test. Differences from control were tested statistically by comparing each treatment group least-square mean with the control group least-square mean using a two-sided Student's t-test, based on the error mean square in the analysis. All statistical tests were two sided. The level of significance was set at $p \leq 0.05$ or at $p \leq 0.001$.

2. **Indices:**

   a. **Reproductive indices:** The reproductive indices were not calculated.

   b. **Offspring viability indices:** The viability (survival) indices were not calculated.

3. **Positive and historical control data:** Positive control data on brain morphometry in pups (MRID 46336204) and on FOB, motor activity and morphometry in adult rats (MRID 46336203) are under review.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality/clinical findings and functional observations:** Intercurrent deaths included two control females with difficulties in parturition. They were found pregnant during post mortem examination. One female in the high dose group which failed to litter by day 25 was sacrificed; this female had only one large fetus. In addition, one mid-dose female that littered one day late (on day 23) was sacrificed due to clinical signs of hunched posture, pinched-sides, piloerection, pallor, feeling cold and weight loss on lactation day 3; the whole litter died by day 3. There were no treatment-related clinical findings noted during general observations or from the functional observational battery on GDs 10 and 17 and lactation days 2 and 9.

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

TABLE 2. Selected mean (±SD) maternal body weight[a]

Observations/study interval	Dose (mg/kg/day)			
	0	0.01	0.05	0.4
Gestation (n= 30)				
Body wt. GD 1 (g)	259.0±17.6	257.8±14.3	263.0±17.6	260.8±16.2
Body wt. GD 7 (g)	294.6±15.9	290.1±17.5	296.9±17.0	294.8±15.8
Body wt. GD 14 (g)	331.0±17.9	328.0±20.2	333.3±17.4	331.9±17.0
Body wt. GD 22 (g)	399.8±26.0	394.3±25.8	403.5±27.8	407.0±25.7
Wt. gain GDs 7-22 (g)[b]	105.17±15.5	104.27±16.7	106.57±19.7	112.27±19.7
Wt. gain GDs 1-22 (g)[b]	140.83±17.6	136.57±19.51	140.43±22.25	146.23±20.13
Lactation (n=30)				

TABLE 2. Selected mean (±SD) maternal body weight[a]				
Observations/study interval	Dose (mg/kg/day)			
	0	0.01	0.05	0.4
Body wt. lactation day 1(g)	303.0±32.6	301.7±29.6	317.1±27.1	308.3±24.0
Body wt. lactation day 5 (g)	316.9±25.7	317.0±24.4	327.0±24.3	325.6±23.4
Body wt. lactation day 7 (g)	322.2±25.6	315.0±20.1	329.8±24.7	326.0±20.5
Body wt. lactation day 15 (g)	352.8±20.2	346.4±20.2	356.4±25.7	357.3±22.6
Body wt. lactation day 22 (g)	363.2±19.6	355.0±21.7	366.4±19.3	360.9±19.3

[a] Data obtained from Tables 3 & 4 pages 70-74, MRID 46153202.
b Calculated by the reviewer

3. **Reproductive performance:** There were no treatment-related effects on pregnancy rate or gestation length (Table 3). All dams littered on day 22 with the exception of one female from the mid dose group which delivered on day 23.

TABLE 3. Reproductive Performance				
Observation	0 mg/kg/day	0.01 mg/kg/day	0.05 mg.kg/day	0.40 mg/kg/day
Number assigned (pregnant)	30 (28)	30 (30)	30 (30)	30 (29)
Gestation length (days)	22.0 ± 0.0	22.0 ± 0.0	22.0 ± 0.2	22.0 ± 0.0
Number of live litters born	28	30	30	29
Number killed with dystocia	2	0	0	1

Data obtained from Table 5, p. 75, MRID 46153202.

4. **Maternal post mortem results:** Dams were not examined at scheduled sacrifice.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during post PNDs 1-5 are summarized in Table 4. Pup survival after PND 5 was not summarized. There was no treatment-related effect on the proportion or percentage of pups born live, number of stillborn, litter size, or proportion of male pup distribution. The whole litter losses (on days 2, 3, or 4) showed no dose-response relationship and were considered to be unrelated to the treatment.

TABLE 4. Litter size and viability [a]

Observation	Dose (mg/kg/day)			
	0	0.01	0.05	0.4
Number of Litters	28	30	30	29
Total number of pups born	347	355	352	361
Number of pups born alive	342	335	331	355
Number of pups born dead	5	20	21	6
Live birth index	98.3±3.8	95.3±15.9	94.5±15.9	98.6±3.6
Number of pups alive, precull (Day 5)	304	266	273	303
Whole litter losses (days 1-5)	1	5	3	3
Proportion of pups surviving on day 5[b,c] (% survival[d])	304 /342 (88.9)	266/335 (79.4)	273/331 (82.5)	303/355 (85.4)
Mean Litter size [c]				
Day 1	12.1±3.1(27)	11.6±2.8 (25)	11.4±2.6 (26)	12.0±2.9 (26)
Day 5 [c]	11.3±3.4 (27)	10.6±2.6 (25)	10.1±3.2 (26)	11.7±2.7 (26)
Sex distribution (% male)				
Day 1	184/342 (53.8)	175/335 (52.3)	173/331 (51.9)	192/355 (55.1)
Day 5 [c]	166/304 (55.7)	139/266 (52.2)	139/273 (51.2)	170/303 (56.6)

[a]Data obtained from Table 6-10, pages 76-80, MRID 46153202.
[b]Includes total litter losses; [c] Before standardization (culling). [d]EPA reviewer calculated the percent survival as the study author reported pup survival (day 1-5) excluding total litter losses in the calculations (Table 9, p79 of the study report)
[c] Excludes total litter losses

2. **Body weight:** Body weight was comparable at birth across all dose groups, and there were no differences in body weight or body weight gain following adjustment for initial weight. Selected mean pre-cull and post weaning pup body weight data are presented in Table 5.

   There were no treatment-related adverse effects on body weight of pups during pre- and post-weaning periods. The body weight of female pups from the mid- and high-dose groups was significantly higher than the controls on PND 5 (pre-cull). For female pups in the high-dose group, body weight remained statistically significantly higher than controls until termination.

TABLE 5. Mean (±SD) pup body weight and body weight gain (g) [a]

PND	Dose (mg/kg/day)							
	0	0.01	0.05	0.4	0	0.01	0.05	0.4
	Males				Females			
1	6.0±0.7	6.0±0.8	6.1±0.06	6.1±0.6	5.7±0.7	5.7±0.7	5.8±0.6	5.8±0.6
5[b]	9.3±2.0	9.8±1.6	10.3±1.6	10.1±1.1	9.0±1.9	9.3±1.4	9.7±1.8	9.7±1.2
Weight gain Days 1-5[b]	3.3±1.3	3.7±1.0	4.0±1.2	3.9±0.8	3.3±1.2	3.5±0.9	3.8±1.3	3.9±0.8
5[c]	9.3±1.7	9.3±1.2	10.3*±1.5	10.1±1.1	8.8±1.6	9.0±1.1	9.9*±1.6	9.7*±1.2
8[d]	14.4±2.3	14.6±1.9	15.7±2.2	15.8±1.5	13.5±2.3	14.0±1.7	15.0±2.3	15.2±1.5
16	34.1±3.2	33.7±3.7	34.8±4.0	35.3±2.6	32.3±3.1	32.7±3.3	33.7±3.8	34.1±2.3
22	52.8±3.9	52.3±5.0	53.7±5.2	55.1±3.7	50.2±3.9	50.6±4.1	52.0±5.1	53.0±3.2
63	345.5±16.5	341.9±21.2	347.3±24.2	355.2±19.0	209.7±16.8	217.8±13.2	218.5±14.6	225.0±14.6

[a] Data obtained from Tables 11 and 15, pages 81-82 and 122-127, MRID 46153202.
[b] Before standardization (culling).
[c] After standardization (post-culling)
d Selected pups received test solution dosed by gavage from post partum day 8 to day 22.

3. **Clinical observations:** There were no treatment-related effects noted on pups during clinical observations.

4. **Developmental landmarks:** The mean age of attainment of preputial separation in males and of vaginal opening for females was unaffected by treatment. Although the mean day of vaginal opening was slightly late compared to controls in the mid-dose group, in the absence of any effect at the high dose, this finding was considered incidental. For mid- and high-dose females, body weight at attainment was greater than that of the control group reflecting the higher body weight observed for these treated groups throughout the study. The data are presented in Table 6.

TABLE 6. Mean (±SD) age of sexual maturation (days) [a]

Parameter	Dose (mg/kg/day)			
	0	0.01	0.05	0.4
N (M/F)	22/22	20/20	22/22	23/23
Preputial separation (males) Body weight at landmark (g)	44.2±1.3 213.5±8.2	44.3±1.0 212.6±13.4	44.5±1.6 213.6±13.8	44.1±1.5 218.7±14.3
Vaginal opening (females) Body weight at landmark (g)	35.4±1.2 122.6±9.3	35.7±1.9 125.1±12.3	37.1*±3.4 133.5**±17.8	36.0±2.4 131.5*±13.3

[a] Data obtained from Table 16, pages 128-129, MRID 46153202.

5. **Behavioral assessments:**

   a. **Clinical findings/Functional observational battery:** Among pups selected for the F_1 generation, six (one control male, one male and two females from the mid dose group and one male and one female from the high dose group) which were sacrificed during the study exhibited one or more of the following signs: paleness, cold to touch, thinness, weight loss, irregular breathing, or found moribund. On day 36 one high dose male was

sacrificed due to a damaged hind limb. There were no treatment-related effects on animals at any dose level on any FOB test day (PND 5, 12, 22, 36, 46, or 61).

b. **Motor/locomotor activity:** No treatment-related motor or locomotor activity effects were noted. The locomotor activity was statistically increased or decreased from controls for one or more treated groups during some intervals on days 14, 22 and 60 for males and females. Habituation was apparent for most groups of males and females on PND 14 and males of different treatment groups on PND18, 22, and 60. The overall motor activity results were highly variable for both sexes on PND14 and 18 and less variable for PND 22 and 60 animals. Total activity data are presented in Table 7.

TABLE 7. Mean (±S.D.) motor activity data (total activity counts for session) [a]

Test Day	Dose (mg/kg/day)			
	0	0.01	0.05	0.4
Males (N= 10-12/dose)				
PND 14	150.1±115.9	143.1±111.2	154.7±92.4	170.0±99.3
PND 18	150.3±81.1	170.1±177.6	139.6±133.5	88.4±85.3
PND 22	341.9±183.9	435.5±214.5	342.6±134.2	342.1±122.7
PND 60	503.7±94.9	586.5±89.0	512.3±120.0	557.6±88.4
Females (N=10-11/dose)				
PND 14	202.7±134.1	184.8±169.1	222.5±167.8	157.8±158.0
PND 18	197.1±155.8	181.3±173.2	152.8±139.8	211.4±137.7
PND 22	402.6±104.0	479.6±192.9	335.0±130.2	439.6±136.9
PND 60	594.1±34.1	629.2±65.4	601.1±43.6	625.3±39.4

[a] Data obtained from Table 17, pages 130-137, MRID 46153202.

c. **Auditory startle:** There were no treatment-related effects in males or females on startle amplitude or time to maximum amplitude on day 23 or on day 61. The statistically significant increases or decreases from control values found mostly in the intermediate dose groups were considered incidental because of lack of dose-response and because they were isolated occurrences. There appear to be a decreased startle response in high dose treated PND 61 females compared to controls but the results are not statistically significant. Habituation was evident as decrease in peak amplitude over successive trial block on each testing day. Peak amplitude data are summarized in Table 8 and time to maximum amplitude data are summarized in Table 9.

TABLE 8. Startle amplitude data (mean Vmax ±S.D.) [a]

Day of test	Trial Block	Dose (mg/kg/day)			
		0	0.01	0.05	0.4
Males (N=11/dose/group)					
PND 23	1-10	315.3±98.6	334.1±117.5	446.1*±234.2	390.2±55.8
	11-20	265.4±141.0	220.8±68.7	279.6±86.3	298.7±89.1
	21-30	212.6±108.4	185.4±66.1	260.9±88.0	255.1±87.6
	31-40	196.6±94.6	169.0±65.6	247.2±78.9	250.6±84.0
	41-50	182.6±70.2	173.8±79.4	260.7*±69.7	209.7±63.7
PND 61	1-10	1698.2±444.5	1211.8*±346.2	1601.1±598.7	1432.7±425.0
	11-20	1096.9±304.9	978.8±375.0	1253.7±402.6	1079.9±357.3
	21-30	1062.1±406.7	851.2±299.1	1010.5±315.0	1007.3±348.4
	31-40	881.4±404.7	730.5±249.2	957.6±254.1	821.7±307.2
	41-50	878.2±263.7	780.0±265.1	1010.3±354.9	693.0±303.8
Females (N= 9-11/dose group)					
PND 23	1-10	500.8±79.2	480.3±165.9	438.2±204.1	370.3*±102.1
	11-20	303.8±79.7	289.4±78.7	304.5±99.7	317.9±102.2
	21-30	312.9±85.1	238.9*±75.5	256.3±55.9	270.1±74.1
	31-40	293.1±85.6	224.2*58.9	232.4±74.4	247.5±69.0
	41-50	265.0±83.6	227.8±71.8	244.5±86.4	210.9±58.5
PND 61	1-10	1130.3±604.7	1395.9±486.6	1137.6±262.1	1020.8±229.8
	11-20	1031.1±476.2	1155.9±361.2	985.2±311.3	916.5±269.6
	21-30	974.4±445.0	976.0±259.0	944.0±369.8	742.3±267.1
	31-40	840.4±455.2	841.2±366.9	748.8±309.5	620.1±218.5
	41-50	825.9±498.5	856.7±273.8	743.1±312.1	638.1±223.6

[a]Data obtained from Table 18, pages 138-141, MRID 46153202.

TABLE 9. Time to Maximum Amplitude data (mean msec ±S.D.) [a]

	Trial Block	Dose (mg/kg/day)			
		0	0.01	0.05	0.4
Males (11/dose/ group)					
PND 23	1-10	24.8±4.1	28.4±6.3	28.3±7.6	25.0±4.4
	11-20	22.8±4.5	22.3±3.7	23.4±5.6	21.1±3.4
	21-30	21.4±2.4	23.6±6.7	22.2±2.8	20.1±2.3
	31-40	20.9±2.5	22.2±3.9	21.6±4.3	20.5±1.9
	41-50	21.3±1.9	22.3±2.7	20.3±2.4	20.4±2.5
PND 61	1-10	26.6±7.4	24.3±5.4	24.7±3.9	24.6±3.9
	11-20	21.0±1.8	23.5±4.5	21.8±3.4	21.7±2.9
	21-30	21.7±2.1	22.8±3.1	21.7±2.0	22.3±2.3
	31-40	22.0±2.3	22.9±3.2	22.8±3.1	23.5±3.2
	41-50	21.9±2.4	24.2±4.3	22.0±2.9	24.2±3.4
Females (9-11/dose//group)					
PND 23	1-10	28.5±7.5	28.7±5.6	29.8±10.8	26.2±6.5
	11-20	24.9±5.1	21.0*±1.3	21.2*±3.9	22.7±4.8
	21-30	21.7±2.6	19.8±0.9	21.2±5.2	23.0±4.7
	31-40	21.9±3.6	19.8±1.0	21.1±4.2	21.6±3.8
	41-50	21.3±3.5	20.5±1.3	19.6±1.4	21.0±2.5
PND 61	1-10	24.1±3.2	24.5±5.1	23.3±2.4	22.3±2.0
	11-20	22.6±2.9	21.6±4.0	22.1±1.3	21.6±1.7
	21-30	21.5±3.7	20.4±2.3	21.5±2.6	24.1*±3.2
	31-40	24.0±5.1	21.8±2.6	22.0±3.3	23.7±2.3
	41-50	23.2±3.6	22.4±3.1	22.1±3.2	23.5±2.6

[a]Data obtained from Table 19, pages 142-145, MRID 46153202.

d. **Learning and memory testing:** There were no treatment-related effects, and acquisition and retention were appropriate in control animals. The performance of selected F1 animals in a 'Y'-shaped water maze was assessed on days 24 or 59 (learning phase) and on days 27 or 63 (memory phase). The performance data are presented as mean straight channel swim time as well as mean water maze time per trial (Table 10) and the mean 'percentage of successful trials' where a successful trial was one completed in less than a specified cut off time, 3-10 seconds or a multiple of that individual rat's straight channel time. (Table 11).

Learning in all dose groups at both time points was demonstrated by a decrease in time taken to complete the Y maze task between the learning phase (on days 24 or 59). Acquisition of learning was demonstrated by the time taken to complete the trial 6 which was approximately half the time taken on trial 1. Memory was demonstrated by the difference in time taken to complete trial 1 between the learning and memory phase. The group mean time for trial 1 in the memory phase was much less than trial 1 in the learning phase for all groups at all time-points. An occasional statistically significant difference from the control group was seen in the mid- and high-dose groups and was not dose-related and showed no consistent trends.

TABLE 10. Learning and Memory Swimming Times (s) (mean ± S.D.) [a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	0.01	0.05	0.4
Males (18-21/dose group)					
Day 24 (Learning)	Straight channel	4.22±1.54	3.76±1.45	4.38±1.84	3.70±1.44
	Trial 1	14.09±6.80	15.78±7.07	16.03±8.14	13.15±7.01
	Trial 2	9.10±6.74	9.19±6.18	8.55±6.87	7.72±5.97
	Trial 3	8.93±6.60	8.35±6.37	5.72±2.68	6.64±6.48
	Trial 4	7.27±4.71	6.11±3.33	7.08±5.98	5.72±2.80
	Trial 5	6.03±3.72	5.10±2.98	6.00±4.84	4.83±2.97
	Trial 6	5.79±2.57	5.01±2.22	5.76±3.75	6.07±3.08
Day 27 (Memory)	Straight channel	2.76±1.32	2.60±0.66	3.59±3.08	2.89±1.24
	Trial 1	6.32±3.36	7.49±4.89	5.25±1.85	6.88±3.22
	Trial 2	5.61±3.65	6.21±5.09	4.58±2.13	5.84±4.63
	Trial 3	5.26±4.58	4.62±2.80	5.36±3.80	5.72±3.91
	Trial 4	4.25±2.33	4.07±2.15	5.44±3.76	4.24±2.23
	Trial 5	4.18±2.54	5.24±2.89	4.69±3.19	5.21±3.60
	Trial 6	4.30±2.20	3.62±2.12	4.67±3.51	3.77±1.63
Day 59 (Learning)	Straight channel	3.72±1.23	3.66±1.51	3.74±1.41	3.40±0.85
	Trial 1	14.01±4.99	13.07±3.99	11.19*±3.72	10.21**±4.09
	Trial 2	7.39±4.60	5.14*±2.07	5.08*±2.68	7.03±3.56
	Trial 3	5.76±2.22	4.56±2.24	4.80±3.07	4.81±2.67
	Trial 4	5.50±3.59	4.07±1.64	4.16±2.37	4.50±2.02
	Trial 5	4.42±2.73	4.78±2.70	3.62±1.13	4.31±1.73
	Trial 6	4.13±1.96	4.92±2.92	4.27±1.84	4.15±1.47
Day 62 (Memory)	Straight channel	2.91±1.14	2.81±0.59	3.19±0.83	3.07±1.25
	Trial 1	6.51±4.01	4.94±2.93	5.02±2.50	4.64±2.09
	Trial 2	4.27±2.05	5.60±3.30	6.48±5.49	4.45±2.91

TABLE 10. Learning and Memory Swimming Times (s) (mean ± S.D.) [a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	0.01	0.05	0.4
	Trial 3	8.60±6.38	6.62±4.98	7.23±5.39	7.23±3.70
	Trial 4	7.41±4.38	6.04±4.78	9.67±5.29	7.06±4.96
	Trial 5	6.28±3.73	6.08±2.89	8.07±4.31	7.29±4.20
	Trial 6	7.01±4.57	7.01±4.14	5.82±2.91	4.87±2.28
Females (N=16-22/dose group)					
Day 24 (Learning)	Straight channel	4.35±1.83	4.32±2.37	4.46±1.81	3.86±1.86
	Trial 1	14.02±5.07	17.78±8.68	15.87±6.65	12.34±5.74
	Trial 2	6.97±4.21	8.22±6.98	8.67±4.75	8.38±6.57
	Trial 3	7.57±4.82	5.66±2.93	6.39±5.45	7.37±5.11
	Trial 4	5.56±3.32	6.28±4.44	8.10±6.38	5.20±2.91
	Trial 5	5.65±3.64	5.29±2.61	5.07±2.75	4.73±2.12
	Trial 6	5.38±2.68	5.18±2.37	5.28±2.48	4.64±3.14
Day 27 (Memory)	Straight channel	3.23±1.32	3.44±2.07	3.40±1.10	2.96±1.25
	Trial 1	6.67±2.86	8.12±5.21	9.58±6.26	6.59±4.43
	Trial 2	5.56±4.15	3.48±1.61	4.55±3.11	5.36±4.71
	Trial 3	4.11±1.71	5.67±4.62	3.88±1.88	4.86±3.90
	Trial 4	4.06±2.73	5.38±2.62	3.83±1.37	5.28±4.03
	Trial 5	5.02±3.98	4.80±1.91	5.35±3.33	4.70±2.50
	Trial 6	4.92±2.57	5.30±4.36	3.72±1.14	4.17±2.54
Day 59 (Learning)	Straight channel	3.98±2.04	3.27±0.82	3.46±1.15	3.27±0.90
	Trial 1	15.39±6.35	12.59±4.31	13.47±6.30	14.99±7.41
	Trial 2	7.22±3.54	5.83±2.18	5.08*±1.71	7.13±3.24
	Trial 3	5.67±4.21	5.12±4.08	4.39±2.26	4.55±2.32
	Trial 4	4.45±2.27	4.33±2.07	2.97*±0.67	4.19±1.97
	Trial 5	4.75±3.42	3.89±1.79	3.55±1.22	3.96±2.12
	Trial 6	5.15±5.07	4.04±2.02	3.08*±0.92	4.33±1.89
Day 62 (Memory)	Straight channel	2.96±1.12	2.86±0.85	3.15±1.61	3.19±1.97
	Trial 1	4.73±2.06	4.84±2.85	5.17±4.75	5.33±2.87
	Trial 2	4.92±2.58	4.36±3.12	4.45±3.50	5.04±3.40
	Trial 3	4.61±3.09	7.05±5.94	6.73±4.64	4.85±2.55
	Trial 4	8.38±4.37	4.76**±1.92	7.53±4.20	6.10*±2.93
	Trial 5	7.01±4.57	7.07±4.83	7.77±3.64	8.09±4.23
	Trial 6	6.72±3.77	5.45±1.94	5.83±3.93	7.44±5.85

[a] Data obtained from Table 20, pages 146-153, MRID 46153202.

TABLE 11. Learning and Memory Percentage of Successful Trials (Mean ± S.D.) [a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	0.01	0.05	0.4
Males (18-21/dose group)					
Day 24 (Learning)	Cut-off 3 sec	5.6±12.2	8.8±17.0	6.1±8.3	11.9±12.0
	Cut-off 4 sec	22.2±18.5	23.7±23.1	26.3±21.7	36.5*±20.2
	Cut-off 5 sec	39.7±22.0	36.8±21.2	41.2±25.7	47.6±22.5
	Cut-off 6 sec	49.2±23.3	50.0±21.5	53.5±23.9	53.2±22.1
	Cut-off 7 sec	52.4±21.3	60.5±20.9	63.2±17.2	63.5±18.7
	Cut-off 8 sec	59.5±23.3	66.7±17.6	65.8±16.2	67.5±18.6
	Cut-off 9 sec	63.5±21.5	70.2±16.3	70.2±14.2	72.2±16.9
	Cut-off 10 sec	68.3±19.7	73.7±18.7	77.2±14.9	77.8±14.3
Day 27 (Memory)	Cut-off 3 sec	34.1±24.4	28.9±22.1	22.8±24.3	29.4±24.7
	Cut-off 4 sec	53.2±23.9	50.9±27.5	50.9±25.1	54.0±24.1
	Cut-off 5 sec	65.9±23.3	63.2±21.2	69.3±23.1	63.5±23.9
	Cut-off 6 sec	74.6±22.1	73.7±19.5	73.7±21.0	69.0±25.4
	Cut-off 7 sec	78.6±19.1	79.8±18.1	83.3±13.6	78.6±19.8
	Cut-off 8 sec	83.3±14.9	81.6±18.3	86.8±10.5	82.5±16.2
	Cut-off 9 sec	88.9±12.2	87.7±14.5	89.5±10.0	84.9±17.4
	Cut-off 10 sec	93.7±8.3	91.2±11.6	93.0±10.1	88.9±14.3
Day 59 (Learning)	Cut-off 3 sec	10.3±14.4	20.4±25.3	18.3±22.2	14.0±18.6
	Cut-off 4 sec	38.9±24.3	45.4±31.2	53.3±27.4	41.2±27.4
	Cut-off 5 sec	51.6±23.5	53.7±25.9	63.3±22.7	55.3±26.1
	Cut-off 6 sec	58.7±23.3	64.8±26.1	72.5±17.3	63.2±25.2
	Cut-off 7 sec	66.7±20.4	70.4±20.3	75.0±15.8	74.6±16.1
	Cut-off 8 sec	70.6±17.4	75.0±18.3	79.2±13.1	81.6*±14.6
	Cut-off 9 sec	74.6±14.5	79.6±13.5	82.5±12.7	84.2*±11.8
	Cut-off 10 sec	81.0±14.2	82.4±10.7	87.5±13.1	89.5*±12.7
Day 62 (Memory)	Cut-off 3 sec	17.5±19.3	23.1±21.5	16.7±19.5	23.7±21.0
	Cut-off 4 sec	36.5±25.1	40.7±26.3	35.0±29.6	40.4±22.4
	Cut-off 5 sec	48.4±26.8	47.2±21.6	46.7±27.9	53.5±25.2
	Cut-off 6 sec	57.9±23.9	62.0±22.7	53.3±25.7	63.2±23.9
	Cut-off 7 sec	65.1±22.3	75.9±17.4	60.0±23.8	69.3±21.0
	Cut-off 8 sec	70.6±22.3	78.7±17.0	67.5±20.6	74.6±22.5
	Cut-off 9 sec	77.0±17.9	82.4±10.7	73.3±22.6	82.5±18.0
	Cut-off 10 sec	82.5±13.4	84.3±9.0	76.7±17.4	86.0±17.8
Females (N=16-22/dose group)					
Day 24 (Learning)	Cut-off 3 sec	10.0±16.6	10.4±17.1	9.6±17.8	14.4±18.0
	Cut-off 4 sec	28.3±24.8	27.1±27.8	29.8±27.0	35.6±24.8
	Cut-off 5 sec	38.3±23.0	41.7±25.8	43.0±21.0	49.2±23.8
	Cut-off 6 sec	49.2±21.9	57.3±20.2	46.5±23.3	54.5±21.9
	Cut-off 7 sec	63.3±23.3	62.5±22.4	55.3±22.3	65.9±20.2
	Cut-off 8 sec	68.3±19.4	69.8±20.4	61.4±15.6	70.5±17.0
	Cut-off 9 sec	70.8±17.0	74.0±18.2	68.4±15.6	73.5±17.6
	Cut-off 10 sec	75.8±16.6	78.1±15.8	71.9±13.7	76.5±18.3
Day 27 (Memory)	Cut-off 3 sec	25.8±24.5	30.2±32.3	23.7±25.6	30.3±21.6
	Cut-off 4 sec	50.0±25.9	41.7±30.4	51.8±19.9	57.6±19.7
	Cut-off 5 sec	63.3±20.7	53.1±30.6	68.4±16.6	65.2±19.9

TABLE 11. Learning and Memory Percentage of Successful Trials (Mean ± S.D.) [a]

Test Day/Parameter		Dose (mg/kg/day)			
		0	0.01	0.05	0.4
	Cut-off 6 sec	73.3±19.0	69.8±18.5	78.1±12.5	72.7±17.5
	Cut-off 7 sec	78.3±14.4	80.2±18.5	85.1±11.0	80.3±16.0
	Cut-off 8 sec	85.0±10.7	84.4±11.3	89.5±11.4	84.8±14.5
	Cut-off 9 sec	89.2±9.8	88.5±10.0	91.2±11.6	86.4±15.1
	Cut-off 10 sec	91.7±10.1	90.6±8.5	92.1±11.6	87.9±12.8
Day 59 (Learning)	Cut-off 3 sec	13.0±21.0	18.4±18.3	32.4*±26.7	20.8±19.4
	Cut-off 4 sec	43.5±22.2	50.0±24.2	55.9±24.3	45.8±22.9
	Cut-off 5 sec	51.9±22.8	57.0±25.6	66.7±15.6	54.2±20.1
	Cut-off 6 sec	62.0±21.2	64.9±26.6	76.5*±11.9	62.5±14.2
	Cut-off 7 sec	68.5±18.0	71.1±21.4	81.4*±13.0	69.2±15.6
	Cut-off 8 sec	69.4±17.4	77.2±19.4	84.3**±9.3	73.3±12.6
	Cut-off 9 sec	75.0±17.4	80.7±16.0	85.3**±10.0	80.0±11.6
	Cut-off 10 sec	78.7±16.0	86.0±12.7	88.2±7.8	82.5±12.7
Day 62 (Memory)	Cut-off 3 sec	17.6±23.2	26.3±26.8	31.4±24.9	23.3±25.6
	Cut-off 4 sec	39.8±26.3	43.0±24.4	40.2±25.7	40.0±23.8
	Cut-off 5 sec	53.7±18.6	57.9±21.8	53.9±22.5	50.0 ±24.2
	Cut-off 6 sec	63.9±17.4	67.5±19.6	60.8±22.0	56.7±23.8
	Cut-off 7 sec	67.6±15.6	77.2±19.4	68.6±17.6	68.3±21.6
	Cut-off 8 sec	76.9±13.0	80.7±18.6	70.6±19.1	75.0±18.3
	Cut-off 9 sec	79.6±10.8	86.8±8.9	73.5±18.7	80.8±14.6
	Cut-off 10 sec	86.1±8.6	86.8±8.9	79.4±15.1	85.0±12.0

[a] Data obtained from Table 21, pages 154-169, MRID 46153202.

e. **Ophthalmology:** No ophthalmoscopic examinations were done.

6. **Postmortem results:**

a. **Brain weight:** No absolute or relative brain weight differences were noted among males at any dose on PND 12. An apparent increase in absolute but not relative brain weight in low-, mid-, and high-dose females on PND 12 was observed compared to controls. There were no effects on brain weight in males and females sacrificed on PND 63 or on the perfused brain weight of males killed on PND 63. The increases in brain weight of PND 12 females were not considered significant as they are within the brain weights of historical controls in studies performed during the recent three years, i.e., 2000-2002 (absolute brain weight range for PND 12 females: 0.92-1.41 g, n=87, p 216 of the study report). Mean brain weight data are presented in Table 12.

TABLE 12. Mean (±SD) Brain Weight Data in Offspring [a]

Parameter	Dose (mg/kg/day)			
	0	0.01	0.05	0.4
Males (n=9-12/dose/group)				
Day 12				
Terminal body weight (g)	23.2±2.5	22.7±3.5	23.0±4.1	24.1±1.4
Brain weight (g)	1.10±0.04	1.11±0.09	1.10±0.09	1.13±0.04
Brain-to-body weight ratio	4.80±0.43	4.93±0.43	4.84±0.54	4.68±0.26
Day 63				
Terminal body weight (g)	351.1±18.7	341.6±27.5	352.1±21.0	359.8±16.6
Brain weight (g)	1.98±0.07	2.00±0.06	1.99±0.07	2.02±0.05
Brain-to-body weight ratio	0.56±0.02	0.59±0.04	0.57±0.03	0.56±0.02
Day 63 (post-perfusion)				
Terminal body weight (g)	350.3±22.2	357.5±26.9	348.6±30.2	357.2±26.9
Brain weight (g)	2.01±0.09	1.99±0.09	1.99±0.11	2.03±0.09
Brain-to-body weight ratio	0.58±0.05	0.56±0.04	0.57±0.04	0.57±0.05
Females (n=11-12/dpse/group)				
Day 12				
Terminal body weight (g)	21.0±3.1	22.4±2.4	24.0±2.6	24.1±3.4
Brain weight (g)	1.03±0.08	1.08*±0.07	1.09*±0.04	1.12**±0.05
Brain-to-body weight ratio	4.95±0.46	4.86±0.33	4.59±0.44	4.72±0.48
Day 63				
Terminal body weight (g)	210.5±13.4	218.6±19.2	214.3±16.0	222.9±14.1
Brain weight (g)	1.86±0.05	1.87±0.04	1.85±0.04	1.88±0.06
Brain-to-body weight ratio	0.89±0.07	0.86±0.07	0.87±0.06	0.85±0.06
Day 63 (post-perfusion)				
Terminal body weight (g)	209.0±23.4	219.2±19.3	216.6±18.6	228.8±20.4
Brain weight (g)	1.77±0.07	1.81±0.06	1.80±0.09	1.85**±0.06
Brain-to-body weight ratio	0.85±0.08	0.83±0.07	0.84±0.05	0.81±0.07

[a] Data obtained from Table 22, pages 170-172, MRID 46153202
Historical control range:for absolute brain weight: males: 0.798-1.21 (g); females: 0.81-1.24 (g)

b. **Macroscopic examination**: No treatment-related effects were reported for male or female offspring at day 12 or 63. Incidental findings involving thoracic cavity were attributed to dosing errors.

c. **Neuropathology**

1) **Microscopic examination**: No significant treatment-related effects were noted on day 12 or 63 at doses up to 0.4 mg/kg/day. On day 63, the incidence of demyelination in the sciatic nerve of minimal severity in high-dose male rats (8/10) was slightly higher than that of concurrent controls (6/10) and slightly higher than the upper end for range of historical control incidence (7/10). This is a common spontaneous finding in this age and strain of rat. Therefore, this finding was not considered to be treatment related.

2) **Brain Morphometry:** Statistically significant differences from controls were noted in a number of morphological measurements. On day 12, decreases were noted in the height and width of the frontal cortex at level 2 and the thickness of the dorsal cortex at level 4 in males, and in the thickness of the dorsal cortex at level 3 in females. Increases were seen in the width and length of the dentate gyrus at level 4 and in the widths of the dentate gyrus and of the hippocampus overall at level 5, in females only. On day 63, decreases were seen in the total width of the brain at level 4 in males, and in the length of the hippocampus at level 3 as well as width of the thalamus at level 4 in females. The morphometric changes in PND 12 females appear to be treatment related. The morphometric analysis of brain from the low- and mid-dose rats was not submitted to assess the dose-response. Data for control and high dose groups are summarized in Table 13.

TABLE 13. Brain morphology in offspring (mean ± S.D.) [a]

Day/Parameters		Dose (mg/kg/day)	
		0	0.4
Males			
Day 12			
Level 2 -Frontal Cortex:	Height	6.29 ± 0.75	5.41**± 0.63
	Width	4.99 ± 0.52	4.34* ± 0.56
Level 3- Hippocampus –	Length From Midline	3.14 ± 0.24	3.03 ± 0.39
Level 3- Dorsal Cortex 1	Thickness	1.38 ± 0.07	1.35 ± 0.13
Level 4 -Dorsal Cortex	Thickness	1.27 ± 0.09	1.16* ± 0.10
Level 4 -Hippocampus	Width Dentate Gyrus	0.51 ± 0.4	0.51 ± 0.04
	Length Dentate Gyrus	1.40 ± 0.17	1.41 ± 0.14
Level 4 Thalamus -	Width	8.37 ± 0.38	8.26 ± 0.55
Level 4 -Thalamus/Cortex -	Overall Width	13.74 ± 0.57	13.36 ± 0.97
Level 5 -Hippocampus	Width Dentate Gyrus	0.74 ± 0.07	0.77 ± 0.09
	Width Overall	1.40 ± 0.09	1.42 ± 0.16
Cerebellum	Height	3.65 ± 0.16	3.59 ± 0.41
	Length	4.29 ± 0.23	4.12 ± 0.41
Day 63			
Level 2-Frontal Cortex	Height	6.43 ± 0.39	6.30 ± 0.30
	Width	4.76 ± 0.19	4.68 ± 020
Level 3-Hippocampus	Length From Midline	2.60 ± 0.27	2.45 ± 0.27
Level 3-Dorsal Cortex	Thickness	1.27 ± 0.07	1.23 ± 0.12
Level 4-Dorsal Cortex	Thickness	1.36 ± 0.07	1.31± 0.11
Level 4-Hippocampus	Width Dentate Gyrus	0.64 ± 0.07	0.61± 0.05
	Length Dentate Gyrus	1.57± 0.19	1.42 ±0.16
Level 4- Thalamus	Width	8.53 ± 0.33	8.10 ± 0.56
Level 4 - Thalamus/Cortex	Overall Width	14.44 ± 0.35	13.74* ± 0.82
Level 5-Hippocampus	Width Dentate Gyrus	0.71 ± 0.04	0.69 ± 0.05
	Width Overall	1.45 ± 0.05	1.44 ± 0.09
Cerebellum	Height	5.42 ± 0.31	5.31 ± 0.25
	Length	6.83 ± 0.33	6.68 ± 0.30

Day/Parameters		Dose (mg/kg/day)	
		0	0.4
Females			
Day 12			
Level 2-Frontal Cortex	Height	5.44 ± 0.78	5.64 ± 0.74
	Width	4.49 ± 0.53	4.46 ± 0.63
Level 3-Hippocampus	Length From Midline	2.87 ± 0.35	3.07 ± 0.19
Level 3-Dorsal Cortex1	Thickness	1.42 ± 0.09	1.33* ± 0.12
Level 4-Dorsal Cortex	Thickness	1.19 ± 0.09	1.17 ± 0.06
Level 4-Hippocampus	Width Dentate Gyrus	0.49 ± 0.05	0.53* ± 0.03
	Length Dentate Gyrus	1.30 ± 0.06	1.39* ± 0.13
Level 5-Hippocampus	Width Dentate Gyrus	0.70 ± 0.10	0.77* ± 0.05
	Width Overall	1.33 ± 0.13	1.43* ± 0.07
Level 4- Thalamus	Width	7.95 ± 0.42	8.15 ± 0.48
Level 4 - Thalamus/Cortex	Overall Width	12.97 ± 0.79	13.46 ± 0.69
Cerebellum	Height	3.60 ± 0.28	3.71 ± 0.33
	Length	4.05 ± 0.38	4.12 ± 0.33
Day 63			
Level 2-Frontal Cortex	Height	6.16 ± 0.30	6.19 ± 0.23
	Width	4.36 ± 0.28	4.52 ± 0.20
Level 3- Hippocampus	Length From Midline	2.64 ± 0.24	2.35** ± 0.15
Level 3-Dorsal Cortex	Thickness	1.24 ± 0.11	1.26 ± 0.09
Level 4-Dorsal Cortex	Thickness	1.34 ± 0.10	1.32± 0.10
Level 4-Hippocampus	Width Dentate Gyrus	0.61 ± 0.03	0.62± 0.05
	Length Dentate Gyrus	1.54± 0.13.	1.42 ±0.14
Level 4- Thalamus	Width	8.27 ± 0.34	7.92* ± 0.38
Level 4 - Thalamus/Cortex	Overall Width	13.78 ± 0.60	13.40 ± 0.76
Level 5-Hippocampus	Width Dentate Gyrus	0.69 ± 0.04	0.68 ± 0.04
	Width Overall	1.42 ± 0.07	1.42 ± 0.06
Cerebellum	Height	5.10 ± 0.37	5.12 ± 0.17
	Length	6.23 ± 0.30	6.24 ± 0.31

aData obtained from Table 26, pages 179-194, MRID 46153202. *p≤0.05; **p≤0.001.
N= 7-12/dose group for males; 9-11/dose group for females

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that administration of dicrotophos at doses up to 0.4 mg/g/day, to pregnant rats from day 7 of gestation through parturition to day 7 post partum and to pups from day 8 to day 22 post partum, produced no evidence of developmental neurotoxicity. Therefore, the overall NOAEL is 0.4 mg/kg/day, for dams and offspring.

B. **REVIEWER COMMENTS:** In dams, no treatment-related effects on mortality, clinical signs, body weight, body weight gain, or FOB parameters were noted.

In offspring, there were no treatment-related deaths, clinical signs or effects on survival, birth weight, body weight or body weight gain pre- or post-weaning, developmental landmarks, FOB parameters, motor or locomotor activity, acoustic startle response, and learning and memory tests. At necropsy, there were no treatment-related gross lesions.

The brain morphometric analyses revealed changes in the high-dose animals at various levels of the brain including the frontal and dorsal cortex, and hippocampus. For high-dose males, statistically significant decreases in size of the frontal cortex (↓14% in height and ↓13% decrease in width) and dorsal cortex (↓8.7% in thickness) during PND 12 were seen as compared to controls. For high-dose females, increased size of hippocampus at Level 4 and 5 (↑6.9-10% for length and width) was reported during PND 12 as compared controls. Decreased Level 3 hippocampus length (↓10.9%) was seen in high dose treated females during PND 63 as compared to controls. Because of the changes seen at the high dose, morphometric data for all tissues (cortex, hippocampus, and thalamus) for the low and mid dose groups should be evaluated.

The maternal systemic NOAEL is ≥0.4 mg/kg/day (HDT). The maternal LOAEL is not identified.

The offspring systemic LOAEL 0.4 mg/kg/day based on morphometric changes in the brain of male and femaleoffspring on PND 12 . The NOAEL is not established.

C. <u>STUDY DEFICIENCIES:</u> The brain morphometric analyses of rats from the low- and mid-dose groups were not conducted.

APPENDIX

STUDY TYPE: Developmental neurotoxicity study [gavage study]-rat

TEST MATERIAL (PURITY): Dicrotophos (87.6% a.i.)

CITATION: Milburn, G.M. (2003). Dicrotophos: Preliminary developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderly Park, Macclesfield, Cheshire, UK SK104TJ. Study ID RR0883. October 14, 2003. MRID 46153201. Unpublished.

SPONSOR: AMVAC Chemical Corporation

SUMMARY: In a dose range-finding developmental neurotoxicity study (MRID 46153201), dicrotophos (87.6% a.i.; Batch No. 403001B, Ref. KB-80-9) was administered in deionized water to 15 time-mated female Alpk:APfSD (Wistar derived) rats by gavage at dose levels of 0, 0.05, 0.2 or 1.0 mg/kg/day from gestation day (GD) 7 through to lactation day 22, inclusive. Maternal clinical observations, body weight, food consumption (during gestation) were monitored. Pups were examined for the number, survival, clinical condition and body weight. Brain and erythrocyte cholinesterase activities were measured in 5 dams/group and their fetuses on GD 22, in 5 offspring/group on post natal days (PNDs) 2, 8, 15, and 22, and in 5 dams/group on lactation day 22. The objective of this study was to select the dose levels for a definitive developmental neurotoxicity study.

No treatment related effects on maternal clinical observations, body weight, or food consumption were observed. Gestation length, pregnancy rate, pup sex ratio at birth, and pup body weight during lactation were similar between the treated and control groups. The high-dose group had a decrease in the number of pups live born with 5/9 litters containing stillborn pups compared with none in the control group. This resulted in slightly decreased mean litter size in the high-dose group (11.1 pups/litter) compared with that of the control group (13.3 pups/litter). In addition, a total of 11 pups from 4 high-dose litters were found dead during PNDs 1-3 compared with none in the control group.

Dose-related inhibition of brain and erythrocyte cholinesterase activities was observed in dams on GD 22 and lactation day 22 and in fetuses on GD 22 and PND 2. In low-, mid-, and high-dose dams, enzyme activity was significantly ($p \leq 0.05$ or 0.01) inhibited on both days by 33% (lactation day 22 only), 48-57%, and 78-82%, respectively, in brain and by 10-14%, 34-37%, and 50-51%, respectively, in erythrocytes. In male and female fetuses on GD 22, brain enzyme activity was inhibited by 13% (females only), 22-30%, and 54-56% in the low-, mid-, and high-dose groups, respectively, and erythrocyte activity was inhibited by 24-28%, and 44-49%, respectively, in the mid- and high-dose groups. On PND 22, brain enzyme activity was inhibited by 9% in low-dose females, by 14% in mid-dose males, and by 9-13% in high-dose males and females. No inhibition of erythrocyte cholinesterase activity was seen in male or female offspring on PND 22. Beginning on PND 8, no inhibition of brain or erythrocyte cholinesterase activities were observed in the offspring. It must be noted that the offspring did not receive the direct dosing of dicrotophos during lactation in the preliminary study and in part could explain

the lack of inhibition or poor inhibition of cholinesterase activity in pups. Further, the exposure of pups to dicrotophos via milk is not characterized in the study.

The data on cholinesterase inhibition in dams, fetuses and the pups are provided in the Table1.

Table 1 : Parent Female and Fetal/Pup Cholinesterase Inhibition

Time Point	Compartment	Sex	0.05 mg/kg/day	0.2 mg/kg/day	1.0 mg/kg/day
Parent Day 22 Gestation	Brain	Female	NS	48%**	78%**
Parent Day 22 Gestation	Erythrocyte	Female	10%	37%**	51%**
Parent Day 22 Lactation	Brain	Female	33%**	57%**	82%**
Parent Day 22 Lactation	Erythrocyte	Female	14%*	34%**	50%**
Fetus Day22 Gestation	Brain	Male Female	NS 13%*	30%** 22%**	56%** 54%**
Fetus Day22 Gestation	Erythrocyte	Male Female	NS NS	24%** 28%**	49%** 44%**
Pups Day 2 Postpartum	Brain	Male Female	NS 9%*	14%* NS	9%* 13%**
Pups Day 2 Postpartum	Erythrocyte	Male Female	NS NS	NS NS	NS NS

NS = not significantly different from Control
* = Statistically significant difference from the Control group at $p<0.05$ level (Student's t-test, two sided)
** = Statistically significant difference from the Control group at $p<0.01$ level (Student's t-test, two sided)

Based on the results of this study, doses for the definitive developmental neurotoxicity study of dicrotophos in rats were chosen at 0.01, 0.05 or 0.4 mg/kg/day.

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

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R114530

Chemical:	**Fenamiphos**
PC Code:	**100601**
HED File Code	**61200 SRRD CDC**
Memo Date:	**09/11/1996**
File ID:	**DPD228885**
Accession Number:	**412-06-0005**

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