DATA EVALUATION RECORD

DIMETHOATE

Study Type: DOSE-FINDING DEVELOPMENTAL NEUROTOXICITY [NON-GUIDELINE]
MRID 45529701

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

Prepared by
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Task Order No. 02-10

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DATA EVALUATION RECORD
TXR#: 0050139

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

PC CODE: 035001 **DP BARCODE:** D278940
SUBMISSION NO.: S605760

TEST MATERIAL (PURITY): Dimethoate (99.1% a.i.)

SYNONYMS: Phosphorodithioic acid, O,O-dimethyl S-[2-(methyl-amino)-2-oxoethyl] ester

CITATION: Meyers, D.P. (2001) Dimethoate dose finding study in CD rats by oral gavage administration preliminary to developmental neurotoxicity study. Huntingdon Life Sciences, Ltd., Cambridgeshire, England. Laboratory Project No. CHV/068 (Report 000129), October 19, 2001. MRID 45529701. Unpublished

SPONSOR: Cheminova A/S, P.O. Box 9, DK-7620 Lemvig, Denmark

EXECUTIVE SUMMARY: In a preliminary developmental neurotoxicity study (MRID 45529701) Dimethoate (99.1% a.i., batch/lot 20522-00) was administered to 15 female Crl:CD® BR rats per dose by gavage at dose levels of 0, 0.2, 3, or 6 mg/kg bw/day. Ten maternal animals/group were administered the test substance from gestation day (GD) 6 through postnatal day 10; an additional five dams/group were dosed on GD 6-20, inclusive. Two male and two female pups/litter were treated from postnatal day 11 to 21, inclusive. The females treated up to GD 20 were killed three hours after dosing on that day; litter data was assessed and cholinesterase activity determined in maternal and fetal plasma, RBC, and brain. For females allowed to litter, the treated offspring were killed two hours after dosing on postnatal day 21 and cholinesterase activities determined. Statistical analyses were performed for gestation body weight and gestation body weight gain data only.

All animals survived to scheduled termination. During gestation, maternal body weight gains after initiation of treatment were slightly decreased in the mid- and high-dose groups (83-88% and 74-88%, respectively, of the control levels). Lower weight gains in the high-dose group

resulted in significantly ($p \leq 0.01$; 93-94% of control) lower absolute body weights compared with the controls beginning on GD 10. There were no apparent differences in weight gain among groups during lactation; no effects on body weights or body weight gains were observed in the low-dose group for gestation or lactation. The only effect on food consumption was slightly reduced intake in high-dose dams during the treatment interval (approximately 88-91% of the control levels).

Following sacrifice on GD 20, no differences were observed between the treated and control groups for mean numbers of corpora lutea, implantations, live fetuses, resorptions, fetal body weights, fetal brain weight, or fetal sex ratios. For dams allowed to litter and rear their young, no differences were observed between the treated and control groups for pregnancy rate, mean numbers of implantations, or pup sex ratios. Pup survival was reduced in the high-dose group mainly during lactation days 1-4. Pup body weight gain in the high-dose group was also decreased during early lactation, resulting in lower absolute body weights throughout lactation. From post-natal day 11-21, body weight gains by the high-dose pups (treated and untreated) were similar to the control levels.

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

For animals tested on GD 20, plasma cholinesterase activity was decreased 25 and 57% in mid- and high-dose dams, respectively, and 66-79% in mid- and high-dose fetuses; RBC cholinesterase activity was inhibited by 70-96% in mid- and high-dose dams and fetuses; and brain cholinesterase activity was inhibited by 75 and 88% in mid- and high-dose dams, respectively. In addition, brain cholinesterase activity was inhibited by 22-24% and 35-42% in mid and high dose fetuses, respectively. On lactation day 21 male and female pups had approximately 40%, 60-65%, and 42-45% inhibition of plasma, RBC, and brain cholinesterase activities at the mid dose, 60%, 70-80%, and 55-66% inhibition of plasma, RBC, and brain cholinesterase activities at the high dose. Data were not statistically analyzed, and sample sizes were small for some groups, precluding conclusions about relative sensitivity among age groups.

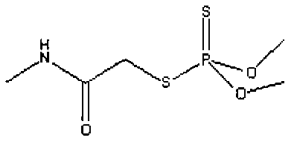
This study is classified Acceptable/Non-guideline as a range-finding study and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); it has provided adequate support for the doses chosen for use in the main developmental neurotoxicity study. These results must be evaluated in context of the developmental neurotoxicity study (MRID 45529703) and the cholinesterase study (MRID 45529702).

COMPLIANCE: Signed and dated GLP, Flagging, and Data Confidentiality statements were provided. A Quality Assurance statement was not included. It was noted that while the study

generally followed GLP principles, no specific study-related Quality Assurance procedures or analysis of dose formulations were performed.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:		Dimethoate
	Description:	white solid
	Batch #:	20522-00
	Purity:	99.1 % a.i.
	Compound Stability:	responsibility of sponsor (a Certificate of Analysis, including stability and storage conditions, was provided)
	CAS # of TGAI:	60-51-5
	Structure:	

2. Vehicle and/or positive control: Reverse osmosis water was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals (P):			
	Species:	rat	
	Strain:	Crl:CD® BR	
	Age at study initiation:	10-11 wks	
	Wt. at study initiation:	215-277 g	
	Source:	Charles River UK Limited, Margate, Kent, England	
	Housing:	In stainless steel or high density polypropylene suspended cages; wood shavings provided as bedding from late gestation onwards	
	Diet:	Pelleted rodent diet, UAR VRF1 Certified, Usine d' Alimentation Rationale, *ad libitum*	
	Water:	tap water, *ad libitum*	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	19-25°C 40-70% up to 15/hr 12 hrs dark/12 hrs light
	Acclimation period:	13 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: May 9, 2000; End: June 24, 2000

2. **Study schedule:** The test substance was administered to ten maternal animals per group from gestation day (GD) 6 through postnatal day 10. An additional five dams/group were dosed on GD 6-20, inclusive. Two male and two female pups/litter were treated from postnatal day 11 to 21, inclusive; the remaining offspring in each litter were retained as undosed within-litter controls. The females treated up to GD 20 were killed three hours after dosing on that day; litter data was assessed and cholinesterase activity determined in maternal and fetal plasma, RBC, and brain. For females allowed to litter, the treated offspring were killed two hours after dosing on postnatal day 21 and cholinesterase activity determined. Dams and untreated offspring were killed on or shortly after postnatal day 21.

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

4. **Animal assignment:** Mated females were allocated to group and cage position in sequence, thus ensuring that animals mated on any one day were evenly distributed among the groups (Table 1). The offspring were allocated to treatment using random number tables.

TABLE 1. Study Design

Treatment schedule	Dose (mg/kg/day)			
	0	0.2	3	6
Maternal Animals (n)				
GD 6-20 (cholinesterase determinations)	5	5	5	5
GD 6 - postnatal day 10	10	10	10	10
Offspring				
Postnatal day 11-21 (cholinesterase determinations)	2/sex/litter	2/sex/litter	2/sex/litter	2/sex/litter

Data taken from text table p. 18, MRID 45529701.

5. **Dose selection rationale:** Dose levels were chosen by the sponsor based on available toxicity data. No further details were included in the current study.

6. **Dosage administration:** All doses were administered once daily to maternal animals by gavage, on either GD 6 through postnatal day 10 or GD 6-20 (animals in parturition at the time of dosing were not dosed on that day). Two offspring/sex/litter were treated on postnatal days 11-21. Dosing volumes for dams and offspring were 5 mL/kg of body weight/day (formulations were mixed with a magnetic stirrer throughout dosing). For dams, dosing was based on the most recent body weight determination up to and including GD 17; thereafter dosing remained constant to postnatal day 1. From postnatal day 1, doses were

again based on the most recently recorded body weight. For offspring, doses were based on the most recently recorded body weight.

7. **Dosage preparation and analysis**: Formulations were prepared weekly. The highest required concentration was prepared by mixing the required amount of test substance with the appropriate amount of vehicle. The formulation was mixed with a magnetic stirrer; lower concentrations were prepared by serial dilution. Prior to the start of the study, homogeneity and stability of the test substance was evaluated as part of the Developmental Neurotoxicity study; these data were not included in the current report. Concentration of the dosing formulations was analyzed in samples from the first and last weeks of the dosing period.

Results:

Homogeneity analysis: data not included

Stability analysis: "Shown to be stable for up to 2 days at ambient temperature or 15 days at 4°C;" data not included.

Concentration analysis: Concentrations of the dosing solutions were within 4.2% of nominal with the exception of the mid-dose solution on week 1, day 1 (10.8% above nominal). Analysis of the mid-dose solution for day 2 showed the concentration to be 99.5% of nominal.

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

C. OBSERVATIONS:

1. In-life observations:

a. Maternal animals: All animals were checked at least twice daily for clinical signs or ill health. Additional, detailed observations were made on each treatment day. Signs of toxicity were recorded as they were observed, including the time of onset, degree, and duration. The FOB was not conducted on the dams.

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

b. Offspring/Litter observations: The day of completion of parturition was designated as lactation day (postnatal day) 0. The females allocated to litter were allowed to deliver their young naturally and rear their own offspring until lactation day 21. Daily throughout

lactation, offspring were examined cage-side for gross signs of mortality or morbidity, changes in litter size, and clinical signs of toxicity. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. Additional, detailed observations were made on each treatment day. Sex of the offspring was determined on lactation days 1, 4, and 21 and individual body weights were recorded on days 1, 4, 7, and 11-21.

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were killed and discarded.

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

2. **Cholinesterase determination:** Cholinesterase activity was determined in blood and brain samples from dams and fetuses on GD 20 and from two male and two female pups/litter which were dosed on lactation days 11-21. Blood was collected under light isoflurane anesthesia from the retro-orbital sinus (dams and 21-day old pups) or umbilical cord (GD 20 fetuses). Samples for GD 20 dams and fetuses were collected 3 hours post-dosing and pooled separately for male and female fetuses in each litter. Blood samples were obtained from 21-day old pups 2 hours post-dosing. Rationale for chosen sampling times was not provided. Following blood collection, the brains were removed and weighed. Plasma, RBC, and whole brain cholinesterase activity was determined by a modified Ellman method.

3. **Postmortem observations:**

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

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

weighed.

In addition, four untreated offspring were killed on lactation day 21 perfused with glutaraldehyde and paraformaldehyde through the left ventricle. Sections of the brain were prepared, stained, and examined by light microscopy.

D. DATA ANALYSIS

1. **Statistical analyses:** Gestational body weight and body weight change data were analyzed by Analysis of Variance. Due to small sample size, other parameters were not analyzed statistically.

2. **Indices:**

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

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

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

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

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

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

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

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

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

II. RESULTS:

A. MATERNAL AND OFFSPRING OBSERVATIONS:

1. **Maternal mortality and clinical and functional observations:**

All animals survived to scheduled termination. Clinical signs of toxicity were limited to post-dosing salivation observed in two mid-dose females on GDs 13 and 15, respectively, in one mid-dose female on lactation day 2, and in three high-dose females on GDs 14, 16, and 19, respectively. These signs may be related to treatment, since they occurred only in animals treated at the mid- and high-doses. However, their toxicological significance is unclear since the sign was sporadic and occurred in very few animals with no increase in incidence from mid to high dose.

2. <u>Maternal body weight and food consumption</u>:

Selected group mean body weight and food consumption data for pregnant and nursing dams are summarized in Table 2. Body weight gains during gestation were slightly decreased in the mid- and high-dose groups (83-88% and 74-88%, respectively, of the control levels). Decreased weight gains in the high-dose group resulted in significantly ($p \leq 0.01$; 93-94% of control) lower absolute body weights compared with the controls beginning on GD 10. There were no differences in weight gain among the treated and control dams during the remainder of treatment (lactation days 1-11). During the post-dosing interval (LD11-21), treated dams tended to gain weight while controls lost weight, resulting in final absolute body weights that were similar for all groups. There were no apparent differences in body weights or body weight gains between the control and low dose groups.

Food consumption in the high-dose group was slightly reduced throughout the treatment interval (approximately 88-91% of the control levels). No other effects on food consumption were observed.

TABLE 2. Selected maternal body weight and food consumption data during gestation and lactation

Study Interval/Endpiont	0 mg/kg/day	0.2 mg/kg/day	3 mg/kg/day	6 mg/kg/day
Gestation (n = 14-15)				
Body wt. GD 0 (g)	247 ± 13	244 ± 15	250 ± 12	241 ± 12
Body wt. GD 6 (g)	289 ± 17	281 ± 17	286 ± 16	275 ± 13
Body wt. GD 14 (g)	337 ± 21	330 ± 23	328 ± 21	314** ± 16 (93)[a]
Body wt. GD 20 (g)	420 ± 26	408 ± 35	400 ± 28	389** ± 24 (93)
Wt. gain GD 0-6 (g)	43 ± 9	37 ± 10	37 ± 8	35 ± 5
Wt. gain GD 6-20 (g)	130 ± 14	127 ± 22	114** ± 14 (88)	114** ± 15 (88)
Food cons. GD 0-2 (g/rat/day)	27 ± 3	26 ± 2	27 ± 3	25 ± 4
Food cons. GD 6-9 (g/rat/day)	31 ± 3	30 ± 3	29 ± 4 (94)	28 ± 4 (90)
Food cons. GD 17-19 (g/rat/day)	33 ± 3	32 ± 4	30 ± 3 (91)	29 ± 3 (88)
Lactation (n = 8-10)				
Body wt. day 1 (g)	329 ± 21	318 ± 18	311 ± 22	299 ± 16 (91)
Body wt. day 11 (g)	365 ± 25	359 ± 17	342 ± 15	331 ± 22 (91)
Body wt. day 21 (g)	348 ± 22	351 ± 32	341 ± 26	340 ± 27 (98)
Wt. gain days 1-11 (g)	36 ± 9	41 ± 8	31 ± 12 (86)	32 ± 12 (89)
Wt. gain days 11-21 (g)	-17 ± 6	-8 ± 17	-1 ± 16	9 ± 12
Food cons. days 1-10 (g/rat/day)	50 ± 4	48 ± 3	49 ± 4	45 ± 6 (90)
Food cons. days 11-20 (g/rat/day)	75 ± 6	75 ± 5	74 ± 4	71 ± 8 (95)

Data taken from Tables 2-7, pp. 52-57, MRID 45529701.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

3. **<u>Reproductive performance and litter observations</u>:** The reproductive performance of animals killed on GD 20 is summarized in Table 3. No differences were observed at any dose between the treated and control groups for mean numbers of corpora lutea, implantations, live fetuses, resorptions, fetal body weights, fetal brain weights, or fetal sex ratios.

TABLE 3. Reproductive performance of females killed on GD 20				
Endpoint	**0 mg/kg/day**	**0.2 mg/kg/day**	**3 mg/kg/day**	**6 mg/kg/day**
No. dams	5	5	5	5
No. with live young	5	5	5	5
Corpora lutea/dam	15.0 ± 1.6	17.2 ± 4.7	16.0 ± 2.5	15.0 ± 1.2
Implantations/dam	15.8 ± 1.1	15.2 ± 1.8	15.2 ± 2.3	14.6 ± 1.5
Live fetuses/dam	15.4 ± 1.5	14.6 ± 1.5	13.6 ± 0.5	14.2 ± 1.3
Total resorptions/dam	0.4	0.6	1.6	0.4
Pre-implantation loss (%)	0.0	12.4	6.1	2.7
Post-implantation loss (%)	2.7	3.8	9.4	2.5
Fetal body wt. (n=5)	3.77 ± 0.25	3.76 ± 0.21	3.79 ± 0.33	3.97 ± 0.25
Fetal brain wt. (n=5/sex) Males Females	 0.169±0.01 0.162±0.01	 0.164±0.01 0.160±0.01	 0.157±0.01 0.156±0.01	 0.172±0.01 0.165±0.01
Sex ratio (% male)	36.9	36.0	48.6	37.1

Data taken from Tables 1, 9, and 10, pp. 51, 59, and 60, respectively, MRID 45529701.

Reproductive and litter data for dams allowed to litter and rear their young are given in Table 4a. No differences were observed between the treated and control groups for pregnancy rate, mean numbers of implantations, total litter size, or pup sex ratios. Pup survival was reduced in the high-dose group mainly during lactation days 1-4 (see Table 4b) resulting in lower live birth, viability, and lactation indices. Also as a result of decreased pup survival, the live litter size on day 1 was reduced for the high-dose group compared with the control group. Pup body weight gain in the high-dose group was decreased during early lactation resulting in lower absolute body weights throughout lactation. Body weight gains by the high-dose pups were similar to the control levels from PND11-21 (for both treated and untreated pups), but did not compensate for the initial reduction.

TABLE 4a. Reproductive performance of females allowed to litter				
Endpoint	**0 mg/kg/day**	**0.2 mg/kg/day**	**3 mg/kg/day**	**6 mg/kg/day**
No. Females	10	10	10	10
Not pregnant	0	1	1	0
Gestation length (days)	22.3	22.1	22.2	22.1
No. live litters	10	9	9	10
Gestation index (%)	100	100	100	100
No. with live at weaning	10	9	9	8
Total litter loss	0	0	0	2
Implants/dam	15.3 ± 1.8	15.2 ± 1.2	15.4 ± 2.1	15.5 ± 1.8
Total litter size (day 1)	14.4 ± 1.6	14.2 ± 2.7	14.3 ± 1.7	14.1 ± 2.0
Live litter size day 1 day 4 (precull) day 11	 14.2 ± 1.5 13.9 ± 1.7 8.0 ± 0.0	 14.2 ± 2.7 14.1 ± 2.7 8.0 ± 0.0	 14.2 ± 1.6 13.8 ± 1.7 7.9 ± 0.3	 12.4 ± 3.5 11.7 ± 4.2 8.0 ± 0.0
Live Birth Index (%)	98.7	100	99.3	87.2
Viability Index (%)	97.8	99.3	96.8	77.5
Lactation Index day 11 (%)	100	100	98.7	88.9
Sex ratio at birth (% male)	52.2	48.3	57.6	52.8
Pup body wt. - male[a] day 1 day 11 day 21	 7.2 ± 0.7 25.1 ± 1.7 52.7 ± 5.4	 6.2 ± 0.7 23.7 ± 3.0 49.4 ± 6.6	 6.6 ± 0.7 25.5 ± 2.4 51.0 ± 4.1	 5.9 ± 0.6 (82)[a] 20.6 ± 3.9 (82) 44.4 ± 8.0 (84)
Pup wt. change - male[a] days 1-11 days 11-21	 18.0 ± 1.7 27.5 ± 3.9	 17.5 ± 2.5 25.7 ± 4.2	 18.9 ± 2.2 25.5 ± 2.7	 14.7 ± 3.6 (82) 23.8 ± 4.4 (87)
Pup body wt. - female[a] day 1 day 11 day 21	 6.8 ± 0.9 23.9 ± 1.5 49.5 ± 4.6	 5.8 ± 0.7 22.1 ± 2.9 46.4 ± 6.1	 6.2 ± 0.5 24.3 ± 2.1 49.0 ± 4.2	 5.9 ± 0.8 (87) 20.4 ± 3.5 (85) 44.5 ± 7.1 (90)
Pup wt. change - female[a] days 1-11 days 11-21	 17.1 ± 1.6 25.6 ± 3.4	 16.3 ± 2.6 24.3 ± 3.5	 18.1 ± 1.9 24.7 ± 2.7	 14.5 ± 2.8 (85) 24.1 ± 4.0 (94)

Data taken from Tables 1, 8, 12-14, 19, 20, 23, and 24, pp. 51, 58, 62-64, 69, 70, 73, and 74, respectively, MRID 45529701.

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

Table 4b. Postnatal Pup Mortality [a]

Dose (mg/kg/day)	Days of Lactation					Total litter loss (day)
	1-4	5-11	1-11	11-21	1-21	
0 (Control)	5(4)	0(0)	5(4)	0	5(4)	0
0.2 (LDT)	1 (1)	0(0)	1(1)	0	1(1)	0
3.0 (MDT)	5 (4)	1(1)	6(4)	0	6(4)	0
6.0 (HDT)	38 (7)	1(1)	39(7)	2(2)	41(7)	2 (2,5)

Number of pups (number of litters); n=9-10 litters.

4. **Postmortem results:** No treatment-related gross lesions were observed in dams killed on GD 20 or in dams and pups killed on lactation day 21. Brain weight data for 21-day old pups are given in Table 5. No differences in brain weight were found among treatment groups.

TABLE 5. Absolute and relative brain weights of 21-day old pups

Weight	0 mg/kg/day	0.2 mg/kg/day	3 mg/kg/day	6 mg/kg/day
Male (n=15-20)				
Body weight (g)	52 ± 4.9	50 ± 6.8	51 ± 4.2	44 ± 8.1
Absolute brain wt. (g)	1.496 ± 0.092	1.462 ± 0.082	1.492 ± 0.046	1.438 ± 0.082
Relative brain wt. (% body wt.)	2.894 ± 0.289	3.000 ± 0.405	2.943 ± 0.218	3.363 ± 0.507
Female (n=14-20)				
Body weight (g)	49 ± 4.5	47 ± 5.7	49 ± 4.2	44 ± 6.9
Absolute brain wt. (g)	1.447 ± 0.061	1.397 ± 0.071	1.468 ± 0.052	1.431 ± 0.073
Relative brain wt. (% body wt.)	2.973 ± 0.257	3.035 ± 0.301	3.019 ± 0.227	3.333 ± 0.438

Data taken from Table 27, p. 77, MRID 45529701.

B. **CHOLINESTERASE ACTIVITY:** Plasma, RBC, and brain cholinesterase activity levels for dams and offspring are given in Table 6. At 6 mg/kg/day, there was substantial inhibition in all compartments for all groups tested. The percent inhibition was fairly consistent for plasma (57-79%) and RBC (70-96%); brain showed somewhat more variability, with less inhibition in fetuses (35-42%) than in pups (55-66%) or dams (88%). Inhibition was also

seen in all compartments at 3 mg/kg/day (25-75% for plasma, 60-82% for RBC, and 22-75% for brain). The only inhibition seen at 0.2 mg/kg/day was in fetuses (12-20% inhibition in plasma, 30% inhibition in RBC [females only]). As noted above, data were not statistically analyzed; small sample sizes may have contributed to some of the variability, especially for dams and fetuses.

TABLE 6. Cholinesterase activities

Tissue	0 mg/kg/day	0.2 mg/kg/day	3 mg/kg/day	6 mg/kg/day
Gestation day 20				
Dams (n=5)				
Plasma (U/L)	1255 ± 180.5	1374 ± 167.6	939 ± 218.6 (25)	545 ± 65.4 (57)
RBC (U/L)	1245 ± 131.6	1205 ± 77.9	275 ± 25.0 (78)[a]	190 ± 28.5 (85)
Brain (U/kg)	12710 ± 1333.9	12680 ± 640.9	3240 ± 411.4 (75)	1580 ± 195.6 (88)
Male fetuses (n=2-5 [plasma], 5 [RBC and brain])				
Plasma (U/L)	233 ± 11.7	186 ± 105.6 (20)	58 ± 81.3 (75)	48 ± 31.9 (79)
RBC (U/L)	930 ± 419.2	1000 ± 237.2	280 ± 253.4 (70)	120 ± 120.4 (87)

Brain (U/kg)	2150 ± 562.4	2320 ± 675.1	1670 ± 564.1 (22)	1390 ± 780.5 (35)
Female fetuses (n=3-5 [plasma], 5 [RBC and brain])				
Plasma (U/L)	239 ± 14.7	210 ± 84.8 (12)	81 ± 69.9 (66)	65 ± 5.0 (73)
RBC (U/L)	1165 ± 543.9	820 ± 282 (30)	215 ± 141.0 (82)	50 ± 111.8 (96)
Brain (U/kg)	1970 ± 288.5	2100 ± 871.8	1500 ± 500.0 (24)	1140 ± 638.7 (42)
Lactation day 21				
Male pups (n=15-20; up to 2/litter)				
Plasma (U/L)	535 ± 56.0	554 ± 88.7	329 ± 44.5 (39)	213 ± 51.2 (60)
RBC (U/L)	1504 ± 283.2	1475 ± 248.4	608 ± 246.7 (60)	447 ± 432.0 (70)
Brain (U/kg)	10555 ± 623.8	9942 ± 614.1	5839 ± 749.6 (45)	4720 ± 1654.5 (55)

Female pups (n=14-20; up to 2/litter)				
Plasma (U/L)	494 ± 39.0	529 ± 81.1	294 ± 48.0 (40)	198 ± 57.5 (60)
RBC (U/L)	1464 ± 381.3	1426 ± 523.5	511 ± 152.2 (65)	288 ± 128.1 (80)
Brain (U/kg)	9338 ± 2709.6	9886 ± 445.2	5414 ± 756.7 (42)	3186 ± 827.3 (66)

Data taken from Tables 28-32, pp. 78-82, MRID 45529701.
[a]Number in parentheses is percent inhibition; calculated by reviewer using %I = [(control - treated)/control] × 100

III. DISCUSSION:

This study was conducted as a range-finding study to select doses for a developmental neurotoxicity study on dimethoate. In the current study, pregnant dams were treated by gavage with 0, 0.2, 3.0, or 6.0 mg/kg/day from GD6 through LD10; 2 pups/sex/litter were then dosed by gavage, using the same dose levels, from LD11-21. Pups and dams were observed for clinical signs, body weight and food consumption were measured, and cholinesterase inhibition was evaluated in brain, plasma, and RBCs on GD20 (dams and fetuses) and on LD21 (pups). Statistical analyses of the data were very limited, so most conclusions below are based on qualitative comparison of the results from various groups.

All three doses were well tolerated by pregnant dams, with only a slight decrease in body weight during gestation at 3.0 mg/kg/day, and a slightly larger decrease in body weight gain along with a slight decrease in absolute body weight at 6.0 mg/kg/day. Cesarean section data showed no changes in litter parameters (including the number of live fetuses and fetal weight) at GD20 for any dose group.

Maternal animals also showed no significant toxicity during lactation, with no apparent differences in body weight or body weight gain among treatment groups. However, there was a decrease in pup survival during early lactation (primarily lactation days 1-4) at 6.0 mg/kg/day, indicating excessive toxicity to pups at that dose. This excessive toxicity appeared to be limited to pre-natal or early lactational exposure; there was no indication of increased toxicity to pups

during the time they were being directly dosed (LD11-21). Pup body weight gain was slightly decreased at the high dose during early lactation, but there was no difference during the direct dosing period, and there was no difference in body weight gain during late lactation when treated and untreated littermates were compared.

Cholinesterase inhibition was seen in all compartments at 3.0 and 6.0 mg/kg/day (22-82% inhibition at 3.0 mg/kg/day; 35-96% inhibition at 6.0 mg/kg/day). At 0.2 mg/kg/day, cholinesterase inhibition was limited to plasma (12-20% inhibition) and RBC (30% inhibition, females only) in fetuses. No inhibition in any compartment was seen in pups (at PND21) or dams (at GD20) dosed with 0.2 mg/kg/day. Cholinesterase inhibition data were not statistically analyzed, and the sample sizes were small for some measurements, so the interpretation of the results at the low dose will not be discussed here.

This study is classified Acceptable/Non-guideline as a range-finding study and does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6). The study does provide adequate support for the doses chosen for use in the main developmental neurotoxicity study. The results from the current study must be evaluated in context of the developmental neurotoxicity study (MRID 45529703) and the cholinesterase study (MRID 45529702).