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DATA EVALUATION RECORD

DIMETHOATE

Study Type: SPECIAL STUDY, CHOLINESTERASE INHIBITION [NON-GUIDELINE]
MRID 45529702

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
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Task Order No. 02-10

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TXR#: 0050139

DATA EVALUATION RECORD

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

PC CODE: 035001 **DP BARCODE:** D278940
SUBMISSION NO.: S 605760

TEST MATERIAL (PURITY): Dimethoate (99.1%)

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

CITATION: Meyers, D. (2001) Dimethoate: Effects on cholinesterase in the CD rat (adult and juvenile) by oral gavage administration. Huntingdon Life Sciences, Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. Doc. No. CHV 070/012226. September 27, 2001. MRID 45529702. Unpublished

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

EXECUTIVE SUMMARY:

In a special neurotoxicity study (MRID 45529702), dimethoate (99.1% a.i., batch/lot # 20522-00) was administered to groups of Crl:CD® (SD) IGS BR rats by gavage at dose levels of 0.0, 0.1, 0.5 or 3.0 mg/kg bw/day. Treatment groups consisted of 9 pregnant dams treated from GD 6 through GD 20 and terminated; 10 pregnant dams treated from GD 6 through PND 10 followed by treatment of 1 male and 1 female offspring/litter on PND 11 through PND 21; groups of 8 untreated dams whose offspring were treated on PND 11. In addition, groups of 16 adult male and female rats were treated with dimethoate for 11 days. Although the study investigated the effect of the test material on developmental criteria such as reproductive performance, gestation, fetal viability, etc., the primary purpose was to determine the effect of dimethoate on blood and brain cholinesterase activities in adult male and female rats, pregnant dams, fetuses, and offspring following both acute and repeated exposures.

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No significant treatment-related effects were found on any reproductive or developmental parameters. In addition, the test material did not increase mortality, or cause clinical signs of toxicity in adult male and female rats, fetuses or offspring at any dose. No histopathology of the nervous system was seen in five offspring examined after PND 60.

For almost all groups of adult animals, pregnant dams, fetuses, and pups, dimethoate doses of 3.0 mg/kg/day significantly decreased the activities of plasma, red blood cell (RBC), and brain cholinesterase following acute or multiple daily doses of dimethoate. Acute doses of 0.5 mg/kg caused no significant effects. Repeated exposure to 0.5 mg/kg caused significant inhibition in brain ChEs in dams, fetuses, and nursing pups. By day 60, all ChE levels had recovered. No consistent difference in sensitivity to ChEI was found following acute or repeated exposures.

For acute exposures:

the LOAEL for brain ChEI is 3 mg/kg (adults and pups of both sexes);
the LOAEL for red blood cell ChEI is 3 mg/kg (adults of both sexes); and
the LOAEL for plasma ChEI is 3 mg/kg (male pups and male adults).

The acute NOAEL for ChEI in all compartments is 0.5 mg/kg for both adults and offspring.

For repeated exposures:

the LOAEL for plasma ChEI is 3 mg/kg/day (adults and offspring of both sexes);
the NOAEL for plasma ChEI is 0.5 mg/kg/day;

the LOAEL for red blood cell ChEI is 3 mg/kg/day (adults and offspring of both sexes);
the NOAEL for red blood cell ChEI is 0.5 mg/kg/day;

the LOAEL for brain ChEI is 0.5 mg/kg/day (adults and offspring of both sexes);
the NOAEL is 0.1 mg/kg/day;

The repeated exposure NOAEL for ChEI is 0.1 mg/kg/day based on brain ChEI in

adults and offspring.

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

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

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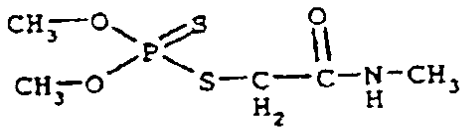
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	Dimethoate
Description:	white solid
Lot/Batch #:	20522-00
Purity:	99.1 % a.i.
Compound Stability:	5 years (stored frozen during study)
CAS # of TGAI:	60-51-5
	

2. Vehicle and/or positive control: purified water/no positive control was used in this study.

3. Test animals (P):	
Species:	rat
Strain:	Crl:CD® (SD) IGS BR
Age and wt. at study initiation:	Virgin Females - 10-11 weeks - 216-260 g; Male and females, 7-8 weeks, males 221-286 g, females 166-210 g
Source:	Charles River UK Ltd., Margate, Kent, England
Housing:	stainless steel or polypropylene cages with wire mesh floors. Wood shavings used for bedding from late gestation onwards
Diet:	Certified UAR VRF1 pelleted rodent diet, Charles River UK Ltd., *ad libitum*
Water:	tap water, *ad libitum*

Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	40-70%
	Air changes:	15/hr
	Photoperiod:	12 hrs light/dark
Acclimation period:	Virgin Females - 9-10 weeks - at least 5 days; Males and females - 5-6 weeks - at least 12 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates** - Start: July 10, 2000 End: October 3, 2000

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

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Table 1. Study Design

Group	Dimethoate Dose (mg/kg/day)	Number of animals/sex	Treatment
1	0	19 F	Nine treated from GD 6 to GD 20; Ten treated from GD 6 to PND 10 with offspring from 8 litters treated from PND 11through PND 21
2	0.1	19 F	Nine treated from GD 6 to GD 20; Ten treated from GD 6 to PND 10 with offspring from 8 litters treated from PND 11through PND 21
3	0.5	19 F	Nine treated from GD 6 to GD 20; Ten treated from GD 6 to PND 10 with offspring from 8 of the litters treated from PND 11through PND 21
4	3.0	19 F	Nine treated from GD 6 to GD 20; Ten treated from GD 6 to PND 10 with offspring from 8 litters treated from PND 11through PND 21
5	0	8 F	No treatment of dams. On PND 11, one male and one female offspring/litter were treated with 0.0, 0.1, 0.5, or 3.0 mg/kg dimethoate.
6	0	16 F/16 M	Eight male and females were treated for one day; eight male and females treated for 11 consecutive days.
7	0.1	16 F/16 M	Eight male and females were treated for one day; eight male and females treated for 11 consecutive days.
8	0.5	16 F/16 M	Eight male and females were treated for one day; eight male and females treated for 11 consecutive days.
9	3.0	16 F/16 M	Eight male and females were treated for one day; eight male and females treated for 11 consecutive days.

Data from pp 22-23 of MRID 45529702

1. **Mating procedure:** Females were paired on a 1:1 basis with stock males of the same strain. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for spermatozoa. The day a vaginal smear tested positive for sperm or at least three copulation plugs were found was designated GD 0.

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

Male and female rats in Groups 6-9 were allocated based on sex and weight (5g blocks). Rats were randomly selected from each block by rotating to compose the treatment groups.

Offspring from mated female rats in Group 5 were assigned to one of the four treatment groups as follows: one male and one female pup from each litter with the lowest within-litter identity numbers for each sex were assigned to the control group; one male and one female pup with the second lowest identity number were treated with 0.1 mg/kg test material; one male and one female pup with the third lowest identity number were treated with 0.5 mg/kg test material; and one male and one female pup with the highest identity number were treated with 3.0 mg/kg test material.

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1. **Dose selection rationale:** Doses were selected by the Sponsor based on a dose-finding study in CD rats (MRID 45529701). The 3.0 mg/kg dose was chosen as the high dose based on reduction of maternal body weight gain during gestation and a decrease in cholinesterase (CHE) activity in dams, fetuses, and offspring. Increased pup mortality was seen at 6 mg/kg.

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

3. **Dosage preparation and analysis:** Formulations were prepared weekly. The highest concentration (0.6 mg/ml) was prepared by adding the required amount of test substance to an appropriate amount of water and mixing with a magnetic stirrer. The 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. Samples were obtained from solutions prepared for use during the first week of treatment and the first week of lactation for determination of test material concentration.

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: All doses were within 1.7% of nominal.

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

C. OBSERVATIONS

1. In-life observations

a. **Adult animals:** All animals were checked at least twice daily for clinical signs or ill health. In addition, gross observations of all rats were made: prior to treatment, as each animal was returned to the cage, at the end of dosing for each group, between 1 and 2 hours after completion of dosing, and as late as possible during the work day.

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

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b. Offspring: The day of completion of parturition was designated as lactation day (postnatal day) 0. Live pups from each litter were counted and weighed individually on PNDs 1, 4, 7, 11, 14, 17, 21, 28, and weekly until study termination on PND 60. Gross observations of all offspring were made on each day of dosing, prior to treatment, as each animal was returned to the cage, at the end of dosing for each group, between 1 and 2 hours after completion of dosing, and as late as possible during the work day. Selected F1 offspring were subjected to weekly full physical examinations from weaning through study termination.

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

2. Termination Schedule and sample collection

Adults and/or offspring were terminated according to the schedule shown in Table 2.

Table 2. Termination Schedule

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

Data from p 27, MRID 45529702

Blood samples were collected from the retro-orbital sinus under light isoflurane anesthesia (all adults and PND 21 pups) or from the umbilical cord (GD 20 fetuses). Blood samples

from fetuses within each litter were pooled prior to analysis. Blood samples from PND 4 and PND 11 pups were collected following decapitation. All blood samples were collected with heparin as the anticoagulant. Samples were packed on water ice and taken to clinical pathology for processing and centrifugation. Resulting samples were stored at -80° C and shipped in dry ice to Huntingdon Research Centre for analysis.

With the exception of PND 4 and PND 11 pups sacrificed by decapitation, all adult and offspring were sacrificed by CO_2 inhalation. The adult and pup brains were removed, weighed, wrapped in aluminum foil and quick frozen in liquid nitrogen. GD 20 fetuses were sacrificed by chilling on a cool plate and the brains were removed, pooled/litter, weighed, and flash frozen in aluminum foil. All samples were stored at -80°C until analysis.

3. Cholinesterase determination

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Cholinesterase assays were done on all red blood cell (RBC), plasma, and brain samples. All cholinesterase assays were done on a Hitachi 911 clinical analyzer. Erythrocyte cholinesterase activity was measured by following the hydrolysis of acetylthiocholine to thiocholine and its subsequent reaction with 6,6'-dithiodinicotinic acid (DTNA) to form a colored product. Although not reported, it is assumed the reaction was monitored at 340 nm. Plasma and brain cholinesterase activity were measured by following the action of thiocholine on 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to form a colored product. Although not reported, it is assumed the reaction was monitored around 410 nm.

4. Necropsy procedures

All animals underwent a detailed macroscopic examination. In addition, the reproductive tract of adult GD 20 females, complete with ovaries, was dissected out and the following recorded: number of corpora lutea in each ovary, number of implantation sites, number of resorption sites (classified as early or late), and the number, distribution, and sex of fetuses in each uterine horn.

Five offspring received an intraperitoneal injection of a barbiturate on PND 61. The heart was exposed *in situ* to permit gravity perfusion with glutaraldehyde and paraformaldehyde via the left ventricle.

Following sacrifice by perfusion, the brain and the sciatic and tibial nerves were removed from five PND 61 pups/group and processed for microscopic examination. Fixation was completed by immersion in glutaraldehyde and paraformaldehyde. 4-5 μm sections of the brain were cut and stained with H&E. 2μm sections of sciatic and tibial nerves were cut and stained with toluidine blue. Examinations included coronal sections of the olfactory lobes, forebrain, cerebrum, hippocampus, thalamus, hypothalamus, tectum, tegmentum, and medulla oblongata, and mid-sagittal sections of the cerebellum/pons. Longitudinal and sagittal sections were prepared of the tibial and sciatic nerves.

A. DATA ANALYSIS

1. Statistical analyses: On parametric data, statistical evaluations were done by ANOVA followed by Williams' test. Nonparametric data were evaluated by the Kruskal-Wallis test followed by Shirley's test. The basic sample unit for litter data was the litter. Where 75% or more of the values for a given variable were the same, Fisher's exact test was used. For all statistical analyses, the level of significance was $p \leq 0.05$.

2. Indices:

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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:

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

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

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

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

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

II. RESULTS

A. Mortality and clinical and functional observations:

All adult animals survived to individual group termination. Pup survival is shown in Table 3. No treatment-related clinical signs of toxicity were observed in adults or in offspring during lactation.

B. Body weight and food consumption:

No treatment-related effects were found on adult male or female rats, fetuses, or offspring for

body weight or body weight gain. Pup weights from treated dams are shown in Table 3.

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

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

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Observation	Dose (mg/kg/day)			
	0.0	0.1	0.5	3.0
GD 20 Cesarean Section				
No. Dams (Litters)	9	9	9	9
Corpora Lutea	16.3 ± 1.9	16.1 ± 1.4	16.3 ± 1.7	16.6 ± 1.6
Implantations	14.8 ± 1.9	16.0 ± 1.7	15.3 ± 1.3	16.2 ± 1.3
Mean Total Resorptions	0.6	2.6	0.8	0.9
Live Fetuses	14.2 ± 2.4	13.4 ± 4.7	14.6 ± 1.6	15.3 ± 1.9

Mean Pre-implantation Loss (%)	8.3	2.1	6.5	3.1
Mean Post-implantation Loss (%)	4.1	16.1	5.2	5.7
Fetal Weight (g)	3.89 ± 0.19	3.94 ± 0.18	3.93 ± 0.31	4.06 ± 0.23

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Natural Delivery				
No. Dams (Litters)	8	10	10	10
Mean Gestation Length (days) b	22.1	22.2	22.4	22.2
Gestation Index (%)	100	100	100	100
Live Litter size Day 1 Day 4 (precull) Day 11	 13.3 ± 1.4 13.3 ± 1.4 8.0 ± 0.0	 14.2 ± 1.9 14.0 ± 2.0 8.0 ± 0.0	 13.3 ± 3.8 13.1 ± 3.8 7.7 ± 0.9	 13.7 ± 2.8 13.6 ± 2.8 7.8 ± 0.4
Pup Deaths Birth (Stillborn) Days 1-4 Days 5-11	 0 0 0	 0 2 0	 0 2 0	 0 1 1

Live Birth Index (%)	100	100	100	100
Viability Index (%)	100	98.5	98.5	99.3
Lactation Index (Day 11) (%)	100	100	100	98.8

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Post-implantation survival index (%)	91.1	88.8	89.6	97.5
Pup Body Wt. (Male)				
Day 1 c	6.6 ± 0.6	6.4 ± 0.5	6.6 ± 1.0	6.7 ± 1.0
Day 4 (precull) c	9.7 ± 1.1	9.6 ± 0.8	10.0 ± 1.1	9.9 ± 1.3
Day 11 c	25.6 ± 2.2	25.7 ± 1.8	25.6 ± 1.5	25.2 ± 3.0
Day 14 d	33.4 ± 2.8	32.8 ± 3.7	33.1 ± 2.0	32.2 ± 3.2
Day 21 d	51.7 ± 5.5	51.3 ± 6.4	51.6 ± 3.1	51.5 ± 5.3
Pup Body Wt. (Female)				
Day 1 c	6.4 ± 0.6	6.1 ± 0.3	6.2 ± 1.0	6.2 ± 1.0
Day 4 (precull) c	9.2 ± 1.0	9.2 ± 0.8	9.4 ± 1.2	9.6 ± 1.5
Day 11 c	24.4 ± 2.4	24.7 ± 1.2	24.5 ± 1.8	24.4 ± 3.1
Day 14 d	32.4 ± 3.0	32.3 ± 1.9	32.1 ± 2.2	31.9 ± 3.9
Day 21 d	49.6 ± 5.1	50.5 ± 3.9	49.8 ± 4.3	50.1 ± 6.2

a Data obtained from pages 74, 75, 76, 78, 79, 80, 81, 83, 85, 89, and 155-159 in MRID 45529702
b Calculated by reviewer. c Includes all pups in litter, prior to direct dosing. d Includes only pups that were directly dosed.

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

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

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

	Dose (mg/kg/day)			
	0.0	**0.1**	**0.5**	**3.0**
GD 20 Fetuses (n = 8)	0.163 ± 0.01	0.161 ± 0.01	0.164 ± 0.01	0.167 ± 0.01
PND 4				
Male (n= 19, 15, 14, 17)	0.402 ± 0.049	0.400 ± 0.027	0.404 ± 0.039	0.399 ± 0.031
Female (n = 13, 16, 12, 16)	0.387 ± 0.026	0.368 ± 0.031	0.381 ± 0.046	0.386 ± 0.024
PND 11 (Group 5) (n = 8)				
Male	1.055 ± 0.065	1.057 ± 0.054	1.065 ± 0.082	1.054 ± 0.081
Female	1.049 ± 0.066	1.035 ± 0.069	1.015 ± 0.060	1.041 ± 0.045
PND 21 (n = 8)				
Male	1.502 ± 0.050	1.484 ± 0.077	1.479 ± 0.055	1.458 ± 0.040
Female	1.459 ± 0.070	1.467 ± 0.059	1.453 ± 0.039	1.436 ± 0.063
PND 60 (n = 8)				
Male	2.013 ± 0.081	1.972 ± 0.101	1.944 ± 0.076	1.992 ± 0.053
Female	1.833 ± 0.064	1.886 ± 0.044	1.859 ± 0.056	1.816 ± 0.076

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[a] Data obtained from pages 77 and 93-96 in MRID 45529702

F. Brain and Nerve Histopathology: No treatment-related lesions were observed in the brain or nerve tissue of five pups examined on PND 61.

G. Cholinesterase Activity: The plasma, RBC, and brain cholinesterase activities of treated adult male and female rats, fetuses, and offspring are shown in Table 5.

1. Acute exposures

In adults, acute exposure to 3 mg/kg caused statistically significant cholinesterase inhibition in plasma in males, in red blood cells in both sexes, and in brain in both sexes. Exposure to 0.5 mg/kg caused slight but statistically significant inhibition in brain in adult males (3.6%). No effects were seen at 0.1 mg/kg.

In pups, acute exposure to 3 mg/kg caused statistically significant cholinesterase inhibition in plasma in males, in brain in both sexes, but no significant effects on ChEs in red blood cells, though in females ChEs were inhibited by 26% in relation to controls. In pups, acute exposure to 0.5 mg/kg caused slight but statistically significant cholinesterase inhibition in brain in males (5.1%), and no statistically significant inhibition in plasma or RBCs. No effects were seen at 0.1 mg/kg.

The statistically significant effects of acute exposures on brain ChEI at 0.5 mg/kg in both adults and pups (4-5%) while treatment related, represent a minimal effect level and are therefore concluded to be an NOAEL.
There were no pronounced or consistent differences seen between pups and adults after acute exposure, in the level of ChEI in any compartment, i.e., sensitivity, and the LOAELs/NOAELs were the same.

2. Repeated Exposures
A. Prenatal Exposures to Dams: Gestation Day 6-20

In dams on Gestation Day (GD) 20, 3 mg/kg/day caused statistically significant cholinesterase inhibition in plasma , red blood cells, and brain. 0.5 mg/kg/day caused statistically significant cholinesterase inhibition only in brain (10%). No effects were seen at 0.1 mg/kg/day.

In fetuses on GD20, 3 mg/kg/day caused statistically significant cholinesterase inhibition in plasma, red blood cells, and brain. 0.5 mg/kg/day caused statistically significant cholinesterase inhibition only in brain (10%). 0.1 mg/kg/day also caused statistically significant brain cholinesterase inhibition (12%).

B. 11 days of exposure-Adults

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3 mg/kg/day caused statistically significant cholinesterase inhibition in plasma in males, in red blood cells in both sexes and in brain in both sexes. 0.5 mg/kg/day caused statistically significant cholinesterase inhibition only in brain (10% males; 13% females). 0.1 mg/kg/day had no effect on ChEI in any compartment.

C. Prenatal and postnatal maternal exposure

In PND 4 pups, maternal exposure of 3 mg/kg/day caused statistically significant cholinesterase inhibition in plasma in females, in red blood cells in males, and in brain in males. Inhibition was considerably less than had been seen in the fetuses on GD20, suggesting recovery and/or a lessening of exposure. At 0.5 mg/kg/day, there was small but statistically significant cholinesterase inhibition in plasma in females (8%); and in brain in males (8%). At 0.1 mg/kg/day, males pups also showed statistically significant brain ChEI (10%).

D. Prenatal, postnatal maternal and 11 days direct exposure

In PND 21 pups, 3 mg/kg/day caused statistically significant cholinesterase inhibition in both sexes in plasma, in red blood cells, and in brain. 0.5 mg/kg/day caused statistically significant cholinesterase inhibition in red blood cells in females (23%) and in brain in both sexes (13% males; 12% females). 0.1 mg/kg/day caused slight but statistically significant cholinesterase inhibition in brain in males (4.1%).

E. Day 60 - 40 days after exposure

No statistically significant differences in males were seen on plasma, RBC, or brain ChEI. In females, slight but statistically significant inhibition in brain ChEI was seen at 3.0 and 0.5 mg/kg/day (both 4%).

Table 5. Plasma, RBC, and Brain Cholinesterase Activity in Adults, Fetuses, and Offspring

of Rats Treated with Dimethoate.

Cholinesterase	Dose (mg/kg/day)			
	0.0	0.1	0.5	3.0
Acute Exposures				
Day 1 Adult Males (Groups 6-9)				
Plasma (U/L)	375 ± 49	387 ± 75 (-3)	364 ± 64 (3)	305* ± 40 (19)
RBC (U/L)	1122 ± 226	1247 ± 203 (-11)	1131 ± 68 (-1)	928* ± 112 (17)
Brain (U/kg)	13,794 ± 247	13,544 ± 802 (2)	13,294* ± 241 (4)	12,131** ± 1096 (12)
Day 1 Adult Females (Groups 6-9)				
Plasma (U/L)	688 ± 132	657 ± 137 (5)	729 ± 82 (-6)	602 ± 131 (12)
RBC (U/L)	1209 ± 168	1128 ± 109 (7)	1106 ± 89 (9)	881** ± 87 (27)
Brain (U/kg)	14,150 ± 555	13,625 ± 445 (4)	13,850 ± 687 (2)	12,106** ± 827 (14)

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Cholinesterase	**Dose (mg/kg/day)**			
	0.0	**0.1**	**0.5**	**3.0**
PND 11 Males (Offspring of Group 5)				
Plasma (U/L)	756 ± 113	748 ± 63 (1)	688 ± 49 (9)	614** ± 76 (19)
RBC (U/L)	1663 ± 279	1634 ± 336 (2)	1597 ± 193 (4)	1544 ± 524 (7)
Brain (U/kg)	6475 ± 244	6363 ± 236 (2)	6144* ± 360 (5)	5375** ± 290 (17)
PND 11 Females (Offspring of Group 5)				
Plasma (U/L)	742 ± 110	700 ± 120 (6)	720 ± 79 (3)	609 ± 93 (18)
RBC (U/L)	1997 ± 620	1647 ± 291 (18)	1894 ± 395 (5)	1475 ± 246 (26)
Brain (U/kg)	6256 ± 195	6350 ± 338 (-2)	6125 ± 298 (2)	5144** ± 532 (18)
Repeated Exposures				
GD 20 Dams (Groups 1-4)				
Plasma (U/L)	1381 ± 169	1216 ± 241 (12)[a]	1184 ± 242 (14)	776** ± 258 (44)
RBC (U/L)	1669 ± 180	1563 ± 224 (6)	1459 ± 278 (13)	709** ± 104 (58)
Brain (U/kg)	12,838 ± 1373	13,044 ± 530 (-2)	11,563* ± 300 (10)	5094** ± 1081 (60)
GD 20 Fetuses (Groups 1-4)				
Plasma (U/L)	258 ± 22	257 ± 26 (0)	239 ± 28 (7)	147** ± 24 (43)
RBC (U/L)	1213 ± 79	1225 ± 98 (-1)	1181 ± 172 (3)	834** ± 183 (31)
Brain (U/kg)	1781 ± 175	1569* ± 173 (12)	1600* ± 136 (10)	1188** ± 164 (33)
Day 11 Adult Males (Groups 6-9)				
Plasma (U/L)	343 ± 33	327 ± 44 (5)	302 ± 36 (12)	215** ± 57 (37)
RBC (U/L)	1094 ± 160	1169 ± 435 (-7)	903 ± 164 (17)	456** ± 240 (58)
Brain (U/kg)	14,100 ± 529	13.988 ± 662 (1)	12,700* ± 548 (10)	7469** ± 2484 (47)
Day 11 Adult Females (Groups 6-9)				
Plasma (U/L)	790 ± 119	949 ± 324 (-20)	770 ± 123 (3)	624 ± 164 (21)
RBC (U/L)	1019 ± 141	991 ± 102 (3)	950 ± 82 (7)	375** ± 123 (63)
Brain (U/kg)	14,869 ± 1400	13,913 ± 446 (7)	12,881**±845 (13)	6188** ± 1078 (58)
PND 4 Male (Offspring of Groups 1-4)				
Plasma (U/L)	612 ± 64	607 ± 62 (1)	588 ± 51 (4)	566 ± 55 (8)
RBC (U/L)	1291 ± 226	1403 ± 204 (-9)	1254 ± 202 (3)	1071** ± 157 (17)
Brain (U/kg)	3137 ± 322	2817* ± 434 (10)	2889* ± 215 (8)	2744** ± 335 (13)

PND 4 Female (Offspring of Groups 1-4)				
Plasma (U/L)	640 ± 49	605 ± 50 (5)	591* ± 41 (8)	576** ± 49 (10)
RBC (U/L)	1260 ± 350	1261 ± 230 (0)	1352 ± 273 (-7)	1088 ± 287 (14)
Brain (U/kg)	2823 ± 310	2941 ± 253 (-4)	2650 ± 287 (6)	2638 ± 269 (7)
PND 21 Male (Offspring of Groups 1-4)				
Plasma (U/L)	506 ± 79	535 ± 68 (-6)	478 ± 29 (6)	307** ± 65 (39)
RBC (U/L)	1638 ± 454	1659 ± 394 (-1)	1494 ± 326 (9)	669** ± 161 (59)
Brain (U/kg)	10,375 ± 207	9944* ± 331 (4)	9044** ± 340 (13)	5675** ± 551 (45)
PND 21 Female (Offspring of Groups 1-4)				
Plasma (U/L)	487 ± 70	507 ± 70 (4)	463 ± 54 (5)	304** ± 53 (38)
RBC (U/L)	1900 ± 587	1619 ± 296 (15)	1466* ± 254 (23)	663** ± 205 (65)
Brain (U/kg)	10,275 ± 376	9906 ± 313 (4)	9019** ± 248 (12)	5956** ± 965 (42)
Post Exposure				

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Cholinesterase	Dose (mg/kg/day)			
	0.0	0.1	0.5	3.0
PND 60 Male (Offspring of Groups 1-4)				
Plasma (U/L)	373 ± 80	369 ± 43 (1)	340 ± 37 (9)	337 ± 33 (10)
RBC (U/L)	1075 ± 86	1100 ± 68 (-2)	1100 ± 73 (-2)	1038 ± 125 (3)
Brain (U/kg)	13,000 ± 450	13,100 ± 411 (-1)	12,988 ± 422 (0)	13,044 ± 756 (0)
PND 60 Female (Offspring of Groups 1-4)				
Plasma (U/L)	907 ± 200	915 ± 198 (-1)	945 ± 232 (-4)	846 ± 189 (7)
RBC (U/L)	1109 ± 149	1119 ± 93 (-1)	991 ± 109 (11)	1044 ± 108 (6)
Brain (U/kg)	13,275 ± 277	12,950 ± 317 (2)	12,738* ± 243 (4)	12,744* ± 586 (4)

Data from pp. 97-110, MRID 45529702
[a] Results in parenthesis are percent inhibition relative to control
* = p≤ 0.05, **p ≤ 0.01

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that treatment with the test material at doses up to 3.0 mg/kg/day did not induce effects on body weight, body weight gain, increase mortality, or produce clinical signs of toxicity to dams, fetuses, offspring, or adult male and female rats. In addition, reproductive performance, gestation and implantation were not affected by treatment in dams, nor were litter size, viability, sex ratio, or post-implantation survival affected. No treatment-related effects were found at necropsy of adults or offspring, brain weights were unaffected by treatment, and no treatment-related lesions were found microscopically in the brain or nerve tissue of pups. Treatment with the test material did decrease cholinesterase activity in dams, fetuses, offspring and adult male and female rats at a dose of 3.0 mg/kg. The study author considered the 23% decrease in RBC cholinesterase activity of female PND 21 offspring to be biologically relevant.

The study author established a NOAEL of 0.5 mg/kg in gestating dams and fetuses and young adult rats based on decreases in cholinesterase activity. For juveniles, the author established a NOAEL of 0.1 mg/kg based on the 23% decrease in RBC cholinesterase activity in female offspring (MRID 45614100, Amendment 1 to MRID 45529702).

B. DISCUSSION AND REVIEWER COMMENTS: This study was conducted to determine the effects of dimethoate on cholinesterase activity in male and female adult, juvenile, and fetal CD rats following oral administration. Treatment with the test material at doses up to 3.0 mg/kg did not adversely affect mortality, body weight, body weight gain, reproductive performance, gestation, the sex ratio, viability, implantation, brain weight, or induce grossly observable lesions at necropsy. In addition, no clinical signs of toxicity were observed from adult male and female rats, juveniles, or fetuses and no adverse effects were observed microscopically in the brain or nerve fibers of juvenile rats.

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However, acute or repeated exposure to dimethoate at 3 mg/kg induced significant decrease in cholinesterase activity in the blood and brain in dams, fetuses, offspring, and adult male and female rats. By day 60 cholinesterase activity in offspring had recovered.

The statistically significant effects of acute exposures on brain ChEI at 0.5 mg/kg in both adults and pups (4-5%) were considered treatment related, but considered a minimal effect level.

There were no pronounced or consistent differences seen in the level of ChEI in any compartment between pups and adults after acute exposure, i.e., sensitivity. The LOAELs/NOAELs were the same for all ages evaluated.

This data set provided tight data with low coefficients of variation for most groups and compartments. This provided sensitivity that resulted in a number of statistically significant findings at levels of inhibition between 5-20%. Other criteria that are relevant to a weight of evidence analysis include dose dependent findings, relation of observed statistically significant inhibition to historical findings of statistical significance for a compartment, and correspondence in findings at different timepoints and groups.

For the repeated exposure data, the brain seemed to be the most sensitive compartment, with a variety of statistically significant effects seen at all doses. Statistically significant findings of 5-10% or so for brain ChEI are not uncommon, based on the lower variability usually seen in this tissue in comparison in general to the blood measures.

Statistically significant effects on brain ChEI following repeated exposures at 0.5 mg/kg were seen in dams and fetuses on GD20, male pups at PND 4, both sexes of pups on PND 21, and in young adults of both sexes. For pups on PND 21 and for adult females, where ChEI was 12-13%, these changes were significant at the $p < 0.01$ level. For other groups, changes were significant at the $p < 0.05$ level, and inhibition varied between 8-10%.

Statistically significant effects on brain ChEI following repeated dosing at 0.1 mg/kg were

also found in fetuses, PND 4 males, and PND 21 males, where the level of ChEI varied between 4-12%. There is no dose dependency between 0.1 and 0.5 mg/kg with respect to the levels of brain inhibition in the fetus and the day 4 pups, but effects from the PND 21 pups were dose dependent (4.1% and 12.8% at 0.1 and 0.5 mg/kg, respectively). The lack of dose dependence for the younger rats suggests that the true threshold is higher, assuming that the best point of departure is where the increasing slope of the dose effect curve begins. For this compartment, then, the weight of the evidence show consistent significant and dose dependent effects of around 10% in both sexes and at most timepoints, leading to the conclusion that 0.5 mg/kg/day is the LOAEL for brain ChEI.

In female pups on PND 21, statistically significant cholinesterase inhibition of 23% in RBCs following repeated dosing at 0.5 mg/kg/day was also seen. This magnitude of RBC ChEI is

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generally regarded as adverse, and was concluded by the investigator to be an LOAEL. Examination of the data revealed that this statistical finding was a result of a relatively high control value (1900 ± 587) in comparison to males (1638± 454), who otherwise had very similar RBC values for all other dose groups. The individual data showed that the high female mean was due to high values in 1 animal that was outside the range seen in the females at the low dose or in the males. It was concluded that this effect should not be regarded as treatment related.

With respect to sensitivity in ChEI following repeated exposure, for dams and fetuses at comparable dose levels , the level of ChEI in RBCs and brain was about half as much in the offspring; while the level of plasma ChEI was the same. Comparing the level of inhibition seen in PND 21 pups exposed for 11 days and young adults exposed for 11 days, in males given 3 mg/kg/day, ChEI levels were quite similar; for females plasma levels in adults (21%) was about ½ of that seen in PND 21 female pups (39%), while brain ChEI at 3 mg/kg/day in adult females (58%) was greater than that in female pups (42%). But, at 0.5 mg/kg/day, levels of brain ChEI were similar for male and female pups and adults (10-13%). Overall, based on these data, it is concluded that there is no consistent evidence of increased sensitivity in young animals with respect to ChEI following repeated dimethoate exposure.

For acute exposures:

the LOAEL for brain ChEI is 3 mg/kg (adults and pups of both sexes);
the LOAEL for red blood cell ChEI is 3 mg/kg (adults of both sexes); and
the LOAEL for plasma ChEI is 3 mg/kg (male pups and male adults).

The acute NOAEL for ChEI in all compartments is 0.5 mg/kg for both adults and offspring.

For repeated exposures:

the LOAEL for plasma ChEI is 3 mg/kg/day (adults and offspring of both sexes);
the NOAEL for plasma ChEI is 0.5 mg/kg/day;

the LOAEL for red blood cell ChEI is 3 mg/kg/day (adults and offspring of both sexes);
the NOAEL for red blood cell ChEI is 0.5 mg/kg/day;

the LOAEL for brain ChEI is 0.5 mg/kg/day (adults and offspring of both sexes);
the NOAEL is 0.1 mg/kg/day;

The repeated exposure NOAEL for ChEI is 0.1 mg/kg/day based on brain ChEI in adults and offspring.

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C. <u>STUDY DEFICIENCIES:</u> The bases of the selection of the times of sample collections should have been provided.

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DATA FOR ENTRY INTO ISIS

Special Study

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
0050139	45529702	special study	rats	up to 11 days	oral	gavage	0.0-3.0	0.0, 0.1, 0.5, 3.0	0.1	0.5	Cholinesterase activity	Maternal
0050139	45529702	special study	rats	up to 60 days	oral	gavage	0.0-3.0	0.0, 0.1, 0.5, 3.0	0.1	0.5	Cholinesterase activity	Offspring