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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

PC 015801

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[Mevinphos]]

Subchronic Oral Study (OPP 82-1; OPPTS 870.3100)

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy Date 2/25/99
Reregistration Branch I, Health Effects Division (7509C)
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Reregistration Branch I, Health Effects Divsion (7509C)

DATA EVALUATION RECORD

STUDY TYPE:Subchronic Oral Toxicity in Rats by Gavage; OPPTS 870.3100 [§82-1]

DP BARCODE: D251794
P.C. CODE: 015801

SUBMISSION CODE: S547036
TOX. CHEM. NO.: 160B

TEST MATERIAL (PURITY): Mevinphos (89.57% a.i.)

SYNONYMS: MRD-88-331

CITATION: Keefe, R. (1992) 90-Day Subchronic Oral Toxicity Study in Rats with Mevinphos (MRD-88-331): 233170B. EXXON Biomedical Sciences, Inc., Los Angeles, CA. Laboratory Project ID 233170B, November 4, 1992. MRID 42588501. Unpublished.

SPONSOR: AMVAC Chemical Corporation, Los Angeles, CA

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 42588501), 10 Crl:CDBR rats/sex/group were administered mevinphos [89.89% a.i. (74.8% alpha isomer, 15.09% beta isomer)] by gavage for 90 days at doses of 0.05, 0.50, 1.0 or 1.5 mg/kg/day in males and 0.01, 0.05, 0.50 or 0.75 mg/kg/day. Due to mortality in the HDT males, the dose was reduced from 1.5 mg/kg/day to 1.0 mg/kg/day on Day 36.

Treatment-related deaths were seen in the 0.5 mg/kg/day (1 female), 1.0 (5 males) and 1.5 mg/kg/day (5 males) groups. Clinical signs of toxicity (pinpoint pupils in males and females, fine tremors in males) were observed consistently in the 1.0 and 1.5 mg/kg/day males and in the 0.5 and 0.75 mg/kg/day females beginning on Day 1. Additional clinical signs (coarse tremors, clear oral and/or ocular discharge, urine staining and soft stool, wet rales and hypoactivity) were observed with increasing incidence as the study progressed in these groups. Pinpoint pupils were observed consistently from Day 15 to the end of the study in the 0.5 mg/kg/day males and sporadically throughout the study in the 0.01 mg/kg/day females and 0.05 mg/kg/day males. These findings are of questionable toxicological significance in the latter two groups since the incidence was low and there were no alterations in plasma or brain cholinesterase.

There were no statistically significant changes in body weight. Overall body weight gain was reduced minimally in the 1.5 mg/kg/day males (8% decrease as compared to control) and 0.75 mg/kg/day females (12% decrease). There was a statistically significant increase in cholesterol in

the 1.0 mg/kg/day males, however the magnitude of the increase (41.5 mg/dL vs 28.8 mg/dL in control) is minimal and of questionable toxicological significance.

For **plasma cholinesterase (ChE)**, at the interim assay (Day 44/45), there was a decrease of 47-52% (as compared to the control value) in males dosed at 0.50 mg/kg/day and above and 22-73% in females dosed at 0.05 mg/kg/day and above. All were statistically significant, except for the 0.05 mg/kg/day females. At the terminal assay, plasma cholinesterase was reduced 46-57% in males dosed at 0.50 mg/kg/day and above and 23-79% in females dosed at 0.05 mg/kg/day and above. All were statistically significant, except for the 0.05 mg/kg/day females. For **RBC ChE**, at the interim assay, there were no statistically significant changes. At the terminal assay, there were statistically significant differences (9-12%) in males dosed at 0.05 mg/kg/day and above. These changes were not considered toxicologically significant due to the magnitude of the difference between treated and control values. There were no statistically significant differences in females. **Brain ChE** was decreased 41-56% in the 0.50 and 1.0 mg/kg/day males and 53-58% in the 0.50 and 0.75 mg/kg/day females. On histopathological examination at necropsy, there was a slight increase in the incidence of hepatocelluar vacuolization in the 1.5 mg/kg/day males.

No Observed Adverse Effect Level (NOAEL) = 0.05 mg/kg/day in males and 0.01 mg/kg/day in females. Lowest Observed Adverse Effect Level (LOAEL) = 0.50 mg/kg/day in males and 0.05 mg/kg/day in females based on clinical signs of toxicity and decreased plasma cholinesterase in males and females and brain cholinesterase in males.

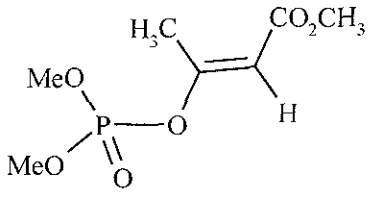
This subchronic toxicity study is classified **Acceptable/Guideline** and does satisfy the guideline requirement for a subchronic oral study (OPPTS 870.1300; OPP 82-1) in rodents.

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

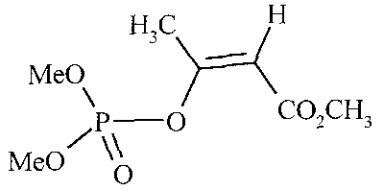
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Mevinphos
   Description: colorless liquid
   Lot/Batch #: 810054, WAF 6-21-89
   Purity: 89.89% ai. (74.8% alpha isomer; 15.09% beta isomer)
   Stability of compound: Stable for 3 yrs when refrigerated (Appendix O)
   CAS #: CAS #: 7786-34-7
   Structure:



Mevinphos - alpha



Mevinphos - beta

2. Vehicle and/or positive control: Reverse osmosis water

3. Test animals: Species: Rat
   Strain: Crl:CDBR (Sprague-Dawley)
   Age and weight at study initiation: 8 wks; males: 247.0-305.4 gms; females: 176.6-221.2 gms
   Source: Charles River Laboratories, Kingston, New York
   Housing: Individually
   Diet: Purina Certified Rodent Chow ad libitum
   Water: Automatic watering system, municipal water ad libitum
   Environmental conditions: Temperature: 68-76°F
   Humidity: 40-70%
   Air changes: Not provided

Photoperiod: 12 hours light/12 hours dark
Acclimation period: 19 days

B. STUDY DESIGN:

1. In life dates - start: December 19, 1989 end: March 23, 1990

2. Animal assignment

Animals were assigned using a computer generated body weight sorting program to the test groups in Table 1.

TABLE 1: STUDY DESIGN (Gavage Administration)

Test Group	Dose Active[a]		Dose as Received[b]		Total Animals	
	M	F	M	F	M	F
1 (Control)	0	0	0	0	10	10
2	0.05	0.01	0.056	0.011	10	10
3	0.50	0.05	0.56	0.056	10	10
4	1.00	.50	1.12	0.56	10	10
5[c]	1.50	0.75	1.67	0.84	10	10

a Dose levels measured in mg of test material/kg of body weight per day
b Measured in mg of test material/kg of body weight per day, includes adjustment for 89.57% active ingredient
c Based on mortality, the active dose for males was decreased from 1.50 (1.67 as received) to 1.00 (1.12 as received) effective Day 36

The study report states that doses were selected based on a 14-day range-finding study and that females were found to be more sensitive than males. No other information was provided.

3. Diet preparation and analysis

The test material was dissolved in reverse osmosis water. Fresh dosing solutions were prepared twice a week and stored refrigerated in the dark. Aliquots were removed for daily administration. No solution was used for more than 5 days. The concentrations of all dosing solutions were measured on a biweekly schedule. The analyses showed that mevinphos concentrations were generally within 10% of nominal, except for three deviations of 11-20% at 2.0 ppm (0.01 mg/kg/day) and 100 ppm (0.5 mg/kg/day).

4. Statistics - The following parameters were statistically analyzed: mean quantitative hematology parameters, mean serum chemistry parameters, mean cholinesterase determinations, mean organ weights, mean organ to body weight ratios and mean body weights, by weighing period. Comparisons were limited to within sex analysis. Standard statistical methods were used. Comparisons were made between Group 4 and 5 males for data collected after Week 7, when the dose level of Group 5 was reduced to that of Group 4. T-tests performed on all quantitative parameters to detect any differences betweent these groups prior to combination showed no differences. Therefore, the combined Group 4 and 5 males results were statistically analyzed and the mean for each parameter was reported at the lower dose.

C. METHODS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality. Initially, clinical observations were conducted immediately after dosing when signs of acute toxicity were expected to be maximal. However, on Day 45, the time of observation was changed to 20-30 minutes post-dosing due to a shift in peak effect, according to the study report.

2. Body weight

Animals were weighed during the week prior to dosing, at dosing initiation (Day 0) and weekly thereafter, on the same day of the week. Body weights were also recorded on the day of sacrifice or on the day of death for animals that died prior to termination.

3. Food consumption

Food consumption was measured weekly during the study on the same day as body weights.

4. Ophthalmoscopic examination

Eyes were examined prior to study initiation and during the final week of the study.

5. Blood was collected from the orbital sinus under methoxyflurane anesthesia for plasma and RBC cholinesterase activity determinations at pretest and during week 7 from non-fasted animals at approximately two to three hours after dosing. On the day of sacrifice, samples were collected from the abdominal aorta after an overnight fast. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
X	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
X	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		Serum osmolarity
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Ornithine decarboxylase		

* Required for subchronic studies based on Subdivision F Guidelines

The method used for cholinesterase determinations was hydrolysis of acetylthicholine and reaction with Ellmans Reagent.

6. Sacrifice and Pathology

Necropsies were conducted on all animals that died or were sacrificed in a moribund condition. Tissues (listed below) were examined grossly and preserved in 10% neutral buffered formalin. After 91 (males) or 92 (females) days, all surviving animals were anesthesized with methoxyfluorane and exsanguinated. Gross necropsies were performed during which the brains were removed from 50% of each group for cholinesterase levels. The remaining 50% were preserved for histopathology. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	X	Brain
X	Salivary glands*	X	Heart*	X	*Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)^{T}
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic n.)^{T}
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	X	Adrenal gland*
X	Colon*	XX	Kidneys*+	X	Lacrimal gland^{T}
X	Rectum*	X	Urinary bladder*	X	Mammary gland^{T}
XX	Liver*$^{-}$	XX	Testes*$^{+}$	X	Parathyroids*$^{++}$
	Gall bladder*		Epididymides	X	Thyroids*$^{++}$
X	Pancreas*		Prostate		
			Seminal vesicle		OTHER
	RESPIRATORY	XX	Ovaries	X	Bone
X	Trachea*	X	Uterus*	X	Skeletal muscle
X	Lung*			X	Skin
	Nose			X	All gross lesions and masses*
	Pharynx				
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
$^{-}$ Organ weight required in subchronic and chronic studies.
$^{++}$ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations :

1. Mortality - A total of 17 deaths occurred prior to the scheduled sacrifice. Six deaths [1 LDT (0.01 mg/kg/day) female, 1 LDT (0.05 mg/kg/day) male, 2 MDT (0.5 mg/kg/day) males and 2 HDT (1.0 mg/kg/day) males] were due to gavage or bleeding. The remaining deaths were in the 0.5 (1 female), 1.0 (5 males) or 1.5 mg/kg/day (5 males) groups and were considered treatment-related. Based on the high incidence of mortality in males, the high dose was reduced from 1.5 mg/kg/day to 1.0 mg/kg/day in the surviving animals on Day 36. The terminal values for the 1.5 and 1.0 mg/kg/day males were combined to run the statistical analyses.

2. Toxicity - Clinical signs of toxicity were observed in the two highest doses in males and females. On Day 1, the following signs were observed: fine tremors in 2/10 males in the 1.5 mg/kg/day group; pinpoint pupils in 1/10 and 4/10 males, respectively, in the 1.0 and 1.5 mg/kg/day groups and in 3/10 and 4/10 females, respectively, in the 0.5 and 0.75 mg/kg/day groups. In the LDT (0.01 mg/kg/day) group females, 3/10 animals had pinpoint pupils on Day 1, but none were observed in the MDT (0.05

mg/kg/day) group. None were reported in the controls. Pinpoint pupils was the most consistently observed clinical sign. Males in the 0.05 mg/kg/day group and females in the 0.01 mg/kg/day group had sporadic observations. Tables 2 and 2a list the other clinical signs and when they were first observed.

Table 2: Clinical Signs in Males

		Dose Levels (mg/kg/day)		
Sign	0.05	0.5	1.0	1.5[a]
Pinpoint pupils	observed sporadically	observed consistently from Day 15 to end of study	Day 1 - observed 1/10 animals at 1.0 mg/kg/day; 4/10 at 1.5 mg/kg/day; continued throughout study	
Fine tremors			observed in 2/10 animals on Day 1 at 1.5 mg/kg/day; continued when dose reduced	
Coarse tremors				observed first on Day 10; ended on Day 35
Clear oral discharge			observed first on Day 17 at 1.5 mg/kg/day; continued when dose reduced	
Urine staining & soft stool			observed first on Day 20 in 1.5 and 1.0 mg/kg/day groups; continued throughout study	
Wet rales			observed in few animals near end of study	
Hypoactivity			observed in few animals near end of study	

a Extracted from Table 2 (pages 43-86) of the study report

b Dose reduced to 1.0 mg/kg/day on Day 36. Survivors from 1.5 mg/kg/day were included in the 1.0 mg/kg/day group for statistical analyses

Table 2a: Clinical Signs in Females[a]

		Dose Levels (mg/kg/day)		
Sign	0.01	0.05	0.5	0.75
Pinpoint pupils	observed sporadically	observed first on Day 16;	observed in 4/10 animals on Day 1; continued throughout study	observed in 3/10 animals on Day 1; continued throughout study
Fine tremors			observed sporadically; more consistent at end of study	observed first on Day 8; continued throughout study
Coarse tremors				observed sporadically
Clear oral & ocular discharge				observed first on Day 46; continued throughout study
Wet rales				observed occasionally near end of study

a Extracted from Table 2 (pages 43-86) of the study report

B. Mean body weight and weight gain:

There were no statistically significant decreases in body weight between the treated and control groups. Mean body weight gain (calculated by the reviewer) showed minimal decreases (12% in the HDT females). Data were presented for both the 1.0 and 1.5 mg/kg/day male groups, whereas the statistics section of the study report states that these groups were combined. Data on body weight and body weight gain are presented in Tables 3 and 4.

Table 3: Body Weight and Body Weight Gain in Males

	Dose Levels (mg/kg/day)				
Body Weight[a] (gms)					
	0	0.05	0.5	1.0	1.5[b]
Day 0	276.6	278.6	278.0	276.5	279.0
Day 7	315.2	320.4	318.4	310.6	313.7
Day 91	566.6	561.2	572.2	568.2[c]	545.2[d]
Body Weight Gain[e] (gms)					
Day 0-7	38.6	41.8	40.4	34.1	34.7(10)[f]
Day 0-91	290	282.6	294.2	291.7	266.2 (8)

a Extracted from Table 3 (pages 88-91) of the study report.
b Dose reduced to 1.0 mg/kg/day on Day 36
c Based on only 3 animals
d Based on only 5 animals
e Calculated by the reviewer
f Percentage decrease from control value.

Table 4: Body Weight and Body Weight Gain in Females

	Dose Levels (mg/kg/day)				
	0	0.01	0.05	0.50	0.75
Body Weight[a] (gms)					
Day 0	201.1	198.5	199.4	200.6	200.2
Day 7	217.2	213.7	211.2	219.1	215.5
Day 91	322.8	320.7	314.0	333.8	307.8
Body Weight Gain[b] (gms)					
Day 0-7	16.1	15.2	11.8	18.5	15.3
Day 0-91	121.7	122.2	114.6	133.2	107.6 (12)[c]

a Extracted from Table 3 (pages 88-91) of the study report.
b Calculated by the reviewer
c Percentage decrease from control value.

C. Food consumption - There was no treatment-related effect on food consumption.

D. Ophthalmoscopic examination - There were no treatment-related effects on the ophthalmoscopic findings.

E. Blood work:

1. Hematology - There was a statistically significant decrease in the mean hematocrit and hemoglobin values of the 0.05 mg/kg/day males. There is no toxicological significance to this finding since there was no dose-response.

2. Clinical Chemistry - There was a dose-related increase in mean cholesterol in both males and females at study termination which was statistically significant only in the 1.0 mg/kg/day males. The data are presented in Table 5.

Table 5: Mean Cholesterol Levels[a]

	Dose Levels (mg/kg/day)								
	Males				Females				
	0	0.05	0.50	1.0[b]	0	0.01	0.05	0.50	0.75
Cholesterol (mg/dL)	28.8	33.0	36.3	41.5**	39.4	40.9	36.1	46.3	48.4

a Extracted from Table 7 (pages 104-105) of the study report.
b Original 1.5 mg/kg/day dose was reduced to 1.0 mg/kg/day on Day 36.

F. Cholinesterase

At the interim measurements, plasma cholinesterase (ChE) was significantly decreased at 0.5, 1.0 and 1.5 mg/kg/day in males and 0.5 and 0.75 mg/kg/day in females. At the terminal sacrifice, plasma, RBC and brain ChE were significantly decreased in the MDT and HDT males; plasma and brain were significantly decreased in the MDT and HDT females. There was a significant decrease in the terminal RBC ChE in the LDT (0.05 mg/kg/day) males, however the magnitude of the change, 9% decrease, was equivocal and not considered toxicologically significant. The data are presented in Table 6.

Table 6: Cholinesterase Levels in Male and Females[a]

	Plasma (IU/L)		RBC (IU/L)		Brain (IU/L)
	Day 44/45	Terminal	Day 44/45	Terminal	
Male Dose Groups (mg/kg/day)					
0	537 ± 124	510 ± 91	6724 ± 817	8986 ± 308	11432 ± 638[e]
0.05	471 ± 84	494 ± 88	7530 ± 723	8171 ± 470** (9%)[b]	11952 ± 497[e]
0.50	283 ± 25** (47%)	275 ± 26** (46%)	6496 ± 1066	7928 ± 417** (12%)	6747 ± 602** (41%)[f]
1.00	285 ± 54 * (47%)[d]	221 ± 31** (57%)	7130 ± 528	7883 ± 55** (12%)	5044 ±331** (56%)[e]
1.5[c]	256 ± 29** (52%)[e]	NR	6372 ± 763	NR	NR
Female Dose Groups (mg/kg/day)					
0	2558 ± 378	3136 ± 399	7966 ± 1106	7428 ± 640	12012 ± 772[e]
0.01	2314 ± 493	2821 ± 531	7684 ± 544	7184 ± 1060	11780 ± 1080[e]
0.05	1995 ± 478 (22%)	2407 ± 693 (23%)	7736 ± 696	7426 ± 1009	12030 ± 254[d]
0.50	1040 ± 175 ** (59%)	984 ± 121** (69%)	7042 ± 674	6384 ±798	5615 ± 259** (53%)[d]
0.75	689 ± 146** (73%)	657 ± 199** (79%)	7104 ± 763	6432 ±1152 (14%)	5024 ± 285** (58%)[e]

a Extracted from Tables 6 and 7 (pages 99-107) of the study report.
b Percent decrease from control value
c Dose was reduced to 1.0 mg/kg/day on Day 36.
d Based on only 4 animals
e Based on only 5 animals
f Based on only 3 animals
* $p<0.05$; ** $p<0.01$

G. Sacrifice and Pathology:

1. Organ weight - There were no statistically significant differences between treated and control groups. The study report states that there was a dose-related increase in mean relative liver weights in both males and females. The data are presented in Table 7.

Table 7: Liver Weights[a]

	Dose Levels (mg/kg/day)								
	Males				Females				
	0	0.05	0.5	1.0	0	0.01	0.05	0.50	0.75
Mean Weight (gms)	13.50	13.76	14.32	14.30	7.64	7.63	7.59	8.45	7.87
Mean Relative Weight (gms)	0.025	0.026	0.026	0.027	0.025	0.025	0.025	0.027	0.027

a Extracted from Table 10 (pages 114-117) of the study report.

2. Gross pathology - Gross necropsy on the 17 animals that died prior to termination revealed lung discoloration, discoloration and abnormal contents of the gastrointestinal tract, staining of the fur and urinary calculi. Single animals had thickened liver, lung tissue consolidation, brain vascularization and thickened pancreas. Two animals had no observable abnormalities.

3. Microscopic pathology - The HDT males had an increased incidence of minimal or slight centrilobular and midzonal hepatocellular vacuolation. The study report states that this pathological change was not of the usual type and therefore may be treatment related. Animals which did not survive to study termination had congestion of various organs, especially the lungs, which the study report indicated was probably due to the pharmacologic activity of the test material. The data on the hepatic microscopic findings are presented in Table 8.

Table 8: Liver Microscopic Findings[a]

	Dose Levels (mg/kg/day)									
	Males					Females				
	0	0.05	0.50	1.0	1.5[b]	0	0.01	0.05	0.50	0.75
Number Examined	10	1	2	7	10	10	1	1	1	10
Vacuolation, hepatocellular, centrilobular/midzonal	0	0	0	0	2	0	0	0	0	0

a Extracted from Table 1, Appendix P (page 270) of the study report.
b Original 1.5 mg/kg/day dose was reduced to 1.0 mg/kg/day on Day 36.

III. DISCUSSION

A. Study Author's Conclusions: The study author concluded that oral administration of mevinphos resulted in mortality, decreased plasma and brain cholinesterase activity, clinical signs of toxicity and/or possible evidence of liver toxicity in males and females at lethal doses greater than 0.50 mg/kg/day. Administration of 0.05 mg/kg/day or lower resulted in no observable effects in male or female rats. A dose of 1.0 mg/kg/day exceeded the Maximum Tolerated Dose and thus dose selection for the 2 year study should be below 1 mg/kg/day.

B. Reviewer's Conclusions: In this 90-day toxicity study (MRID 42588501), 10 Crl:CDBR rats/sex/group were administered mevinphos [89.89% a.i. (74.8% alpha isomer, 15.09% beta isomer)] by gavage for 90 days at doses of 0.05, 0.50, 1.0 or 1.5 mg/kg/day in males and 0.01, 0.05, 0.50 or 0.75 mg/kg/day. Due to mortality in the HDT males, the dose was reduced from 1.5 mg/kg/day to 1.0 mg/kg/day on Day 36.

A total of 17 deaths occurred prior to the scheduled sacrifice. Six deaths [1 LDT (0.01 mg/kg/day) female, 1 LDT (0.05 mg/kg/day) male, 2 LDT (0.5 mg/kg/day) males and 2 HDT (1.0 mg/kg/day) males] were due to gavage or bleeding. The remaining deaths were in the 0.5 (1 female), 1.0 (5 males) or 1.5 mg/kg/day (5 males) groups and were considered treatment-related. Based on the high incidence of mortality in males, the high dose was reduced from 1.5 mg/kg/day to 1.0 mg/kg/day in the surviving animals on Day 36. The terminal data values for the 1.5 and 1.0 mg/kg/day males were combined to run the statistical analyses. Clinical signs of toxicity (pinpoint pupils in males and females, fine tremors in males) were observed in the 1.0 and 1.5 mg/kg/day males and in the 0.5 and 0.75 mg/kg/day females beginning on Day 1. Additional clinical signs (coarse tremors, clear oral and/or ocular discharge, urine staining and soft stool, wet rales and hypoactivity) were observed with the incidence increasing as the study progressed in these groups. Pinpoint pupils were observed consistently from Day 15 to the end of the study in the 0.5 mg/kg/day males and sporadically throughout the study in the 0.01 mg/kg/day females and 0.05 mg/kg/day males. These findings are of questionable toxicological significance in the latter two groups since the incidence was low and there were no alterations in plasma or brain cholinesterase in the group. The terminal RBC cholinesterase was statistically significantly decreased in the 0.05 mg/kg/day males, but there was only a minimal (9%) difference from the control value. See more complete discussion below.

There were no statistically significant changes in body weight. Overall body weight gain was reduced minimally in the 1.5 mg/kg/day males (8% decrease as compared to control) and 0.75 mg/kg/day females (12% decrease). There was a statistically significant increase in cholesterol in the 1.0 mg/kg/day males, however the magnitude of the increase (41.5 vs 28.8 in control) is minimal and of questionable toxicological significance.

For **plasma cholinesterase (ChE)**, at the interim assay (Day 44/45), there was a decrease of

47-52% (as compared to the control value) in males dosed at 0.50 mg/kg/day and above and 22-73% in females dosed at 0.05 mg/kg/day and above. All were statistically significant, except for the 0.05 mg/kg/day females. At the terminal assay, plasma cholinesterase was reduced 46-57% in males dosed at 0.50 mg/kg/day and above and 23-79% in females dosed at 0.05 mg/kg/day and above. All were statistically significant, except for the 0.05 mg/kg/day females. For **RBC ChE**, at the interim assay, there were no statistically significant changes. At the terminal assay, there were statistically significant differences (9-12%) in males dosed at 0.05 mg/kg/day and above. There were no statistically significant differences in females.**Brain ChE** was decreased 41-56% in the 0.50 and 1.0 mg/kg/day males and 53-58% in the 0.50 and 0.75 mg/kg/day females.

The study report stated that there was a dose-related increase in liver weights in males and females (not statistically significant). This was not demonstrated in the mean weight data for absolute weights. Relative weights were increased, although the changes were minimal. There was a slight increase in the incidence of hepatocellular vacuolation in the 1.5 mg/kg/day males (2/10 vs 0/10 in control).

No Observed Adverse Effect Level (NOAEL) = 0.05 mg/kg/day in males and 0.01 mg/kg/day in females. Lowest Observed Adverse Effect Level (LOAEL) = 0.50 mg/kg/day in males and 0.05 mg/kg/day in females based on clinical signs of toxicity and decreased plasma cholinesterase in males and females and brain cholinesterase in males.

B. Study deficiencies - There are no study deficiencies.