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

DATA EVALUATION RECORD

MEVINPHOS

Study Type: §83-5; Combined Chronic/Oncogenicity Study - Rats

Work Assignment No. 1-01-15G (MRID 43088601)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Reregistration Branch 1, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Combined Chronic/Carcinogenicity
OPPTS Number: 870.4300 OPP Guideline Number: §83-5

DP BARCODE: D251794 SUBMISSION CODE: S547036
P.C. CODE: 015801 TOX. CHEM. NO.: 160B

TEST MATERIAL (PURITY): Mevinphos (85.74% a.i., 74.9% alpha and 10.8% beta).

SYNONYMS: 3-[(dimethoxyphosphinyl)oxy]-2-butenoic acid methyl ester; MRD-88-331

CITATION: Plutnick, R.T., (1994). 2-Year Chronic Toxicity/Oncogenicity Study in Rats with Mevinphos (MRD-88-331). EXXON Biomedical Sciences, Inc., East Millstone, NJ., Laboratory Project Id. 233170C, January 3, 1994. MRID 43088601. Unpublished.

SPONSOR: AMVAC Chemical Corporation, 4100 East Washington Boulevard, Los Angeles, CA

EXECUTIVE SUMMARY: In a combined chronic/oncogenicity study (MRID 43088601), mevinphos (85.74% a.i.) was administered orally by gavage 5 days/week to a total of 80 Sprague-Dawley rats/sex/group) at nominal dose levels of 0, 0.025, 0.35, or 0.70 mg/kg/day for approximately 104 weeks. The high-dose females received 0.70 mg/kg/day until day 82 of the study; on day 83 the dose was lowered to 0.60 mg/kg/day due to signs of acute toxicity. Ten (10) rats/sex/dose each were designated for cholinesterase determination, hematology, and clinical chemistry analyses, and an additional 10 rats/sex/dose were terminated at approximately 52 weeks. The animals that provided blood samples were terminated at approximately 104 weeks. The carcasses of rats specified for cholinesterase measurements were discarded without pathological examination.

There were no treatment-related effects noted in food consumption, mean body weights or body weight gains. Palpable masses were observed at a similar incidence in all groups, including the controls. Hematologic, clinical chemistry, urinalysis, and ophthalmological parameters, as well as organ weights, and gross pathology were also similar in the treated and control groups.

The general condition, behavior, and appearance of treated animals at ≥6 hours post-dosing was unaffected by treatment. On days 681, 688, and 695, observations were performed 30 minutes to 6 hours post-dosing. Tremors was the most frequent finding in both high-dose and mid-dose animals. Other signs of cholinesterase inhibition, such as exophthalmus, oral discharge, and/or anogenital staining were also observed in the treated groups, more frequently in the high-dose males. These findings all decreased after 6 hours post dosing.

The high-dose males had more early deaths than expected due to acute toxicity. There were no statistically significant differences in female survivorship. Survival rates at the terminal sacrifice (104 weeks) were, 21, 27, 28, 15% in controls, low-, mid, and high-dose groups males, respectively; and 21, 21, 24, 22% in the corresponding female groups. Survival rates at 96 weeks were 37-51% in all groups.

Cholinesterase activity was decreased in mid- and high-dose animals; the decreases in enzyme activity were generally greater in the females. At the interim sacrifice, plasma cholinesterase activity was decreased in the high-dose (↓57-71%; p≤0.01) and mid-dose animals (↓38-59% statistically significant [p≤0.01] in females only). Decreases (p≤0.05 or ≤0.01) in brain cholinesterase activity were observed in the high-dose (↓53-55%) and in the mid-dose animals (↓27-43%) Erythrocyte cholinesterase was decreased only 6-8% (p=not significant) in the mid- and high-dose groups at this interval.

At 24 months, there were no surviving control females, only 3-4 surviving male controls, and only 2-3 rats/ dose group of the 10 rats/sex/dose pre-selected for determination of cholinesterase activity. The 24 month brain cholinesterase data were therefore not used for enzyme activity comparisons. Plasma cholinesterase activity in the main study high-dose animals was decreased (p≤0.05 or 0.01) at the 3, 6, 12, and 18 month intervals (↓47-71%). In the mid-dose males and females, plasma ChE was statistically significantly decreased 41-51% and 50-67%, respectively. Decreases (p≤0.05 or ≤0.01) in erythrocyte cholinesterase activity were noted in the high-dose males at 6 months (↓9%) and 18 months (↓12%) and in the high-dose females at 3 (↓17%), 6 (↓20%), and 18 months (↓16%). In the mid-dose males, RBC ChE was statistically significantly decreased 6-11% at the 3 and 6 month measurements and 8-15% (not statistically significant) at the 12 and 18 month measurements. The only statistically significant difference in RBC ChE in females was at the 18 month measurement (13%), although the decreases at the other time points were 9-17%. There were no treatment-related changes in ChE in the 0.025 mg/kg/day males or females, except for a statistically significant decrease in RBC ChE at the 18 month time period in the 0.025 mg/kg/day females. However this effect was not considered toxicologically significant due to the magnitude of the change, i.e. a 8% decrease. In general, the changes in RBC ChE were not considered toxicologically significant due to the magnitude of the differences between treated and control groups.

There were no treatment related non-neoplastic lesions detected in the animals at any interval. There were no neoplasms observed at the interim sacrifice. An increased incidence of hepatocellular adenomas was observed in the high-dose males (2.9% treated vs 0% controls). However, the incidence was within laboratory historical control ranges (0-5%). In high-dose females, an increased incidence of hepatocellular adenomas (4.5% treated vs 1.4% controls) as well as hepatocellular carcinomas (1.5% treated vs 0 controls) were observed. The incidence of

hepatocellular adenomas in high-dose females (4.5%) was not within laboratory historical control ranges (0-2%), but was within the published historical ranges cited by the Sponsor (1.4-21.7%; Mc Martin, et al; 1992; Toxicologic Pathology, 20 #2, pages 212-225).

The chronic LOAEL is 0.35 mg/kg/day in males and females based on decreased plasma and brain cholinesterase activity. The chronic NOAEL is 0.025 mg/kg/day in males and females.

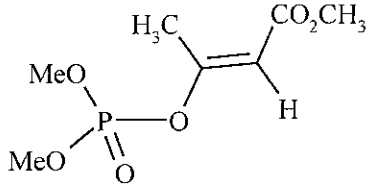
The submitted study is classified as **acceptable/guideline (§83-5)** and does satisfy the guideline requirements for a chronic toxicity study (§83-1) and a carcinogenicity study (§83-2) in rats.

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

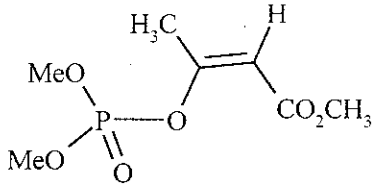
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Mevinphos, technical
   Description: Colorless liquid
   Lot/Batch #: 910072
   Purity: 85.74%, based on 74.94% alpha (α) and 10.80% beta (β) isomers
   Stability of compound: Compound is stable in water for up to 27 days when stored under refrigeration.
   CAS #: 7786-34-7
   Structure:



Alpha Isomer



Beta Isomer

2. Vehicle: Reverse osmosis water

3. Test animals: Species: Rat
   Strain: Crl:CDBR (Sprague-Dawley)
   Age and weight at study initiation: 7 weeks old; 229.4-283.1 g (males) and 164.4-223.7 g (females)
   Source: Charles River Breeding Laboratories, Inc., Kingston Facility, Stone Ridge, New York
   Housing: Suspended stainless steel with wire mesh; 1 rat/cage
   Diet: Purina Certified Rodent Chow 5002 (mash), ad libitum, Manufacturer: Purina Mills, Inc., Richmond, IN
   Water: Tap water, ad libitum

Environmental conditions:
Temperature: 68-76° F
Humidity: 40-70%
Air changes: Not reported
Photoperiod: 12 h dark/12 h light
Acclimation period: 15 days

B. STUDY DESIGN:

1. In life dates - start: 9/26/90 end: 9/30/92.

2. Animal assignment: Animals were assigned (stratified by weight) to treatment groups as indicated in Table 1.

Table 1. Study design

Test Group	Dose M/F (mg/kg/day)	Number of Animals			
		Main Study 24 months		Interim Study 12 months [b]	
		Males	Females	Males	Females
Control	0/0	50	50	30	30
Low	0.025/0.025	50	50	30	30
Mid	0.35/0.35	50	50	30	30
High	0.70/0.60 [a]	50	50	30	30

a Dose level was lowered from 0.70 mg/kg on day 83 due to acute toxicity.
b 10 animals/sex/dose each were used for clinical chemistry analyses, for cholinesterase determinations, and for interim sacrifice at 12 months. Surviving rats from the groups designated for chemistry and cholinesterase determinations were sacrificed after approximately 104 weeks of dosing.

3. Dose selection: The study report stated that dosing levels were based on the results of a 90-day oral toxicity study; however, no further information was provided. In a 90-day oral study in rats (MRID 42588501), the NOAEL was 0.05 mg/kg/day in males and 0.01 mg/kg/day in females. The LOAEL was 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 (ChE) in males and females and brain ChE in males. The clinical sign at these doses was pinpoint pupils in males and females. Plasma ChE was decreased 46-47% in males and 22-23% in females; brain ChE was decreased 53% in males.

4. Dose preparation, administration, and analysis: Dosing solutions were prepared weekly and stored in a dark refrigerator; no solution was used for more than 5 days. Dose was administered by gavage, at a dose volume of 5 mL/kg, 5 days/week for approximately 104 weeks; control animals received reverse osmosis water at a volume of 5 mL/kg. The dose administered was recalculated weekly based on the most recent body weight. Analyses for dose concentration were performed weekly for the first 3 months, and bimonthly thereafter; concentrations used for analysis were 5, 70, 120 (added at day 83 for the high-dose females), and 140 ppm. The study report stated that analysis for homogeneity and stability was performed on both the high- and low-dose groups prior to dose initiation and that homogeneity was reassessed whenever the volume of the dosing preparation was changed or at least every six months during the first 18 months of study. Data submitted with the analytical chemistry report shows that homogeneity analyses were performed at 2, 5, and 140 ppm by analysis of triplicate aliquots taken from a single sample at each concentration and that aliquots of the 2, 5, and 140 ppm concentrations were refrigerated and tested at intervals up to 27 days for subsequent stability analysis.

   Results - Homogeneity analysis: 2 ppm, 2.08±0.02 ppm; 5 ppm, 5.08±0.01 ppm; 140 ppm, 139±2.6 ppm; each had a coefficient of variation (CV) of <2%.

   Concentration analysis: 5 ppm, 5.11±0.24 ppm; 70 ppm, 70.5±2.3 ppm; 120 ppm, 120±4.0 ppm; 170 ppm, 140±4.9 ppm.

   Stability analysis: Concentrations were as follows: 2 ppm, 102.0-106.0% of nominal; 5 ppm, 98.8-107.0% of nominal; 140 ppm, 97.9-103.6% of nominal.

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

5. Statistics - Bartlett's test for homogeneity of variance was applied to the body weight, food consumption, cholinesterase activity, hematology, urinalysis, organ weight, and organ to body weight data. Parametric and nonparametric methods were used. A one-way ANOVA followed by the Dunnett's test was applied to the parametric data if significant differences among the means were indicated. For the nonparametric procedures, the Kruskal-Wallis Test was applied followed by Dunn's Summed Rank Test if significant differences among the means were indicated. Liver adenoma and carcinoma incidences and survivorship were analyzed according to Peto et al. (1980).

C. METHODS:

1. Observations: Animals were inspected for viability twice daily (once a day on weekends and holidays). Clinical observations, including palpation for masses, were made weekly. Observations for detailed cholinergenic effects were made on days 681, 688, and 695 post-dosing, which was considered a representative period for these observations,

according to the study report.

2. Body weight: Animals were weighed prior to dosing, on the first day of dosing (day 0), weekly for the first 13 weeks, biweekly in months 4 and 5, every 4 weeks thereafter, and at termination.

3. Food consumption: Food consumption for each individual rat was determined weekly for the first 13 weeks, biweekly in months 4 and 5, and every 4 weeks thereafter.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were performed on all animals prior to dose initiation and prior to termination.

5. Blood analyses: Blood was collected from the first 10 surviving animals/sex/group at 6, 12, and 18 months, and at termination for hematological and clinical analyses. All blood parameters were determined using blood collected from the orbital sinus of animals anaesthetized with methoxyflurane and fasted overnight prior to blood sampling. The CHECKED (X) parameters below were examined in each blood sample.

   a. Hematology:

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)
	Corrected leukocyte count (Cor WBC)	X	Mean corpusc. volume (MCV)
X	Erythrocyte count (RBC)	X	Reticulocyte count
X	Platelet count		Erythrocyte morphology
	Blood clotting measurements		
	(Thromboplastin time)		
	(Prothrombin time)		

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose
X	Carbon dioxide	X	Total bilirubin
		X	Total serum protein
	ENZYMES	X	Triglycerides
X	Alkaline phosphatase (AP)		Albumin/globulin ratio
	Plasma cholinesterase (PL-ChE)		
	Erythrocyte cholinesterase (RBC-CHE)		
	Brain cholinesterase (BR-CHE)		
	Creatine phosphokinase		
	Lactate dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT)		
X	Serum aspartate aminotransferase (AST)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase (GLDH)		

6. Urinalysis: Urinalyses were performed on the first 10 surviving animals/sex/group at 6, 12, 18, and 24 months. The following CHECKED (X) parameters were examined.

	Transparency	X	Glucose
X	Volume	X	Ketones
X	Specific gravity		Bilirubin
	pH	X	Blood
X	Sediment (microscopic)		Color
X	Protein		Urobilinogen
	Osmolality		Chloride
X	Appearance		Creatinine
			Potassium
			Sodium
			Urea

7. Cholinesterase analysis: For plasma and erythrocyte cholinesterase activity, blood was collected prior to the start of dosing, at 3, 6, 12, and 18 months, and at termination from the next-to-the-last 10 animals/sex/dose. Samples were collected within 4 hours of dosing from the main and interim sacrifice groups. Blood was collected from the orbital plexus of non-fasted animals anaesthetized with methoxyflurane. Brain cholinesterase levels were measured in 10 rats/sex/group at the interim and terminal sacrifices. Measurements were done using the Ellman method.

8. Sacrifice and pathology: All animals that died or were killed *in extremis* and those sacrificed on schedule (12 and 24 months) were subjected to gross pathological examination and the CHECKED (X) tissues were collected. Additionally, the (XX) organs were weighed for those animals sacrificed on schedule. Following blood and brain sample collection, carcasses of rats specified for cholinesterase measurements were discarded without further examination.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT		NEUROLOGIC
	Tongue	X	Aorta	XX	Brain (3 levels)
X	Salivary glands	X	Heart	X	Peripheral nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes and optic nerve
X	Jejunum	X	Thymus		GLANDULAR
X	Ileum		UROGENITAL	XX	Adrenal glands
X	Cecum	XX	Kidneys	X	Hardarian gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	X	Parathyroids
XX	Liver	X	Epididymides	X	Thyroid
	Gall bladder	X	Prostate		OTHER
X	Pancreas	X	Seminal vesicles	X	Bone
	RESPIRATORY	XX	Ovaries	X	Skeletal muscle
X	Trachea	X	Oviducts	X	Mandibular lymph nodes
X	Lungs	X	Uterus	X	Skin
X	Nasal cavity	X	Vagina	X	Lacrimal gland
X	Pharynx		Ureter	X	All gross lesions and masses
	Larynx		Urethra		

All tissues collected from the control and high-dose animals at the 104 week terminal sacrifice and from the animals that died or were killed *in extremis* were examined microscopically. At the terminal sacrifice, only the eyes/optic nerve, kidneys, liver, lung, and tissues with gross changes of the mid- and low-dose animals were examined microscopically; at the interim sacrifice, the eyes/optic nerve and liver of the control and high-dose animals were examined.

II. RESULTS

A. Observations:

1. Toxicity - The general condition, behavior, and appearance of treated animals at ≥6 hours post-dosing (recorded on a weekly basis) was considered unaffected by treatment. Low incidences of hypoactivity and/or impaired use of limbs were observed in all groups including the controls. Palpable masses were observed at a similar incidence in all groups, including the controls. It was stated that the female high-dose level was reduced from 0.70 mg/kg/day to 0.60 mg/kg/day on day 83 due to signs of acute toxicity. However, on days 77 through 84 the survival rates for all rats (80/sex/dose) were 99-100%, and the summary tables for clinical signs do not indicate increased incidences of acute toxicity. The only clinical sign of toxicity, which had a minor increased incidence in the mid- and high-dose females, was dried red ocular discharge.

   On days 681, 688, and 695, observations were performed 30 minutes to 6 hours post-dosing. Tremors was the most frequent finding. An increased incidence of tremors was observed in the high-dose males (14-17/24-28 treated vs 0/26 controls) and females (7-11/23-25 treated vs 0/22-25 controls) 30 minutes after dosing; 2-4 high-dose males and 1 high-dose female were still experiencing tremors 6 hours post-dosing. Tremors were also displayed by the 0.35 mg/kg/day males and females at 30 minutes post-dosing (1-3/28-30 treated). Other signs of cholinesterase inhibition, such as exophthalmus, oral discharge, and/or anogenital staining were also observed in the treated groups, more frequently in the high-dose males. These findings all decreased over the 6 hour period.

2. Mortality - There is some confusion about the reporting and calculation of survival rates. The sponsor stated that male and female groups showed adequate survivorship at their scheduled termination. The study report gives the following number (%) of male survivors for the increasing doses: 27/80 (34%), 31/80 (39%), 30/80 (38%) and 22/80 (28%). For females, the corresponding data are 25/80 (31%), 27/80 (34%), 29/80 (36%) and 28/80 (35%). Those rates appear to be based on Table 2 (page 52) of the study report, a portion of which is reproduced below.

Mortality in Males

	Dose Levels (mg/kg/day)			
Test Period	0	0.025	0.30	0.70
Weeks 1-105	53	49	50	58
12 Month Sacrifice	10	10	10	10
Cholinesterase Sacrifice	3	3	3	3
Number Surviving to Terminal Sacrifice	14	18	19	10

Mortality in Females

	Dose Levels (mg/kg/day)			
Test Period	0	0.025	0.30	0.60
Weeks 1-105	55	53	51	52
12 Month Sacrifice	10	10	10	10
Cholinesterase Sacrifice	0	3	3	3
Number Surviving to Terminal Sacrifice	15	14	16	15

If the number at Week 105 is subtracted from 80, this is the number of survivors used to calculate the rates in the study report. However, this is not the true number remaining at the end of the study because 10 animals were lost at the interim sacrifice and some were lost at the cholinesterase sacrifice. The numerator for the survivor rates should be the number of animals surviving to the terminal sacrifice. The denominator should be the total number of animals minus the interim and cholinesterase sacrifices. Therefore, the survival rates at the terminal sacrifice of the 67-70 rats/dose should be 14/67 (21%), 18/67 (27%), 19/67 (28%) and 10/67 (15%) in controls, low-, mid-, and high-dose groups males, respectively; survival rates were 15/70 (21%), 14/67 (21%), 16/67 (24%) and 15/67 (22%) in the corresponding females groups. In general, the survival rates at termination were less than guideline requirement (not less than 25%) for this interval. From the information provided with the ophthalmological examinations (page 194 of study report), survival rates at 96 weeks were 37-51%.

The statistical analysis (Appendix MM, pages 3254-3259) contains a table labeled "Survivorship and Significance Levels for Early Deaths", which gives significant difference and trend. The numbers used for the calculations were the number dead by Week 105 in the above tables. Statistical analysis of male survivorship resulted in a significant difference between groups at **p=0.22** with a dose response significance at **p=0.04.** The data from this table are presented in Table 2.

Table 2. Survivorship and significance levels for early deaths.[a]

Males				
Dose level (mg/kg/day)	0	0.025	0.35	0.70
Observed	53	49	50	58*
Expected	54	56.6	53.5	46
Females				
Dose level (mg/kg/day)	0	0.025	0.35	0.60
Observed	55	53[b]	51	52
Expected	52.5	55.4	54.7	48.4

a These data were extracted from study report Appendix MM, Table 1, page 3256; n=80 rats/sex/dose; the Observed number is the number dead at Week 105.
b Summary tables indicated that 52 deaths occurred in the low-dose females.
* Significant difference between groups at p=0.22 with a dose response significance at p=0.04 .

B. Body weight and body weight gain:

There were no treatment related differences in body weights in the treated groups compared to controls. All of the statistically significant ($p \leq 0.05$ or 0.01) differences from controls were approximately 4% and were judged to be not of toxicological concern.

C. Food consumption:

There were no treatment related differences in mean food consumption (g) in the treated groups compared to controls. There were decreases in food consumption observed in all dose groups compared to controls. These sporadic (↓3-16%) statistically significant ($p \leq 0.05$ or 0. 01) findings were considered not of toxicological concern.

D. Ophthalmoscopic examination: There were no treatment related ophthalmological findings detected in rats dosed with mevinphos for up to 96 weeks. In males, corneal scarring was observed in the controls, low-, mid-, and high-dose groups (8/28, 12/34, 8/31, and 13/28, respectively). It was explained that the lesions may have been due to trauma experienced during blood sampling.

E. Blood analyses:

1. Hematology - Hematological parameters were unaffected by treatment with mevinphos.

2. Clinical chemistry - Clinical chemistry parameters were unaffected by treatment with mevinphos. Alkaline phosphatase activity was increased in the high-dose females at 18 months (↑73%, p≤0.05); activity was also increased (↑61%) at 24 months, but the difference was not statistically significant. Other statistically significant (p≤0.05 or 0.01) differences in clinical chemistry parameters, such as phosphorus and albumin, were considered not to be treatment related since the changes were minor, not sustained throughout the treatment period, and/or did not exhibit a definitive dose-response relationship.

3. Cholinesterase analysis - Cholinesterase (ChE) enzyme activity data are presented in Tables 3a and 3b. At the interim sacrifice (Table 3a), **plasma ChE activity** was significantly decreased (p≤0.01) in the high-dose males (↓57%) and females (↓71%) and in the mid-dose females (↓59%); a decrease in enzyme activity in the mid-dose males (↓38%) was not statistically significant. Decreases (p≤0.05 or ≤0.01) in **brain ChE activity** were observed in the high-dose males (↓53%) and females (↓55%) and in the mid-dose group (males, ↓27%; females, ↓43%). **Erythrocyte ChE** was decreased only 6-8% (p=not significant) in the mid- and high-dose groups at this interval.

   For the main study groups, ChE data at the 3, 6, 12, and 18 months were used for comparison. Because the 10 rats/sex/dose chosen for ChE activity were not replaced if they died, at 24 months, there were no surviving control females, only 3-4 surviving male controls, and only 2-3 rats/dose group. The 24 month brain ChE data were not used for comparisons. **Plasma ChE activity** in the main study high-dose animals was decreased (p≤0.05 or 0.01) at the 3 ,6, 12, and 18 month intervals (males, ↓47-61%; females, ↓66-71%) (Table 3b). In the mid-dose males and females, plasma ChE was statistically significantly decreased 41-51% and 50-67%, respectively. **Erythrocyte (RBC) ChE activity** was also decreased (↓5-20%) at these intervals in the high-dose animals; statistically significant (p≤0.05 or ≤0.01) decreases were noted in the high-dose males at 6 months (↓9%) and 18 months (↓12%) and in the high-dose females at 3 (↓17%), 6 (↓20%), and 18 months (↓16%). In the mid-dose males, RBC ChE was statistically significantly decreased 6-11% at the 3 and 6 month measurements and 8-15% (not statistically significant) at the 12 and 18 month measurements. The only statistically significant difference in RBC ChE in females was at the 18 month measurement (13%), although decreases at the other time points were 9-17%.

   There were no treatment-related changes in ChE in the 0.025 mg/kg/day males or females, except for a statistically significant decrease in RBC ChE at the 18 month time period in the 0.025 mg/kg/day females. However this effect was not considered toxicologically significant due to the magnitude of the change, i.e. a 8% decrease.

Table 3a. Mean values of plasma, brain, and erythrocyte cholinesterase activities detected at the interim sacrifice in rats dosed with mevinphos at 0, 0.025, 0.35, 0.60 or 0.70 mg/kg/day. [a]

Males			
Dose (mg/kg/day)			
0	0.025	0.35[b]	0.70[c]
Plasma Cholinesterase (IU/L)			
639	919	398 (38%)	273** (57%)[d]
Erythrocyte Cholinesterase (IU/L)			
6036	6186	5545 (8)	5644 (6)
Brain Cholinesterase (IU/L)			
11732	11728	8575* (27)	5569** (53)
Females			
Dose (mg/kg/day)			
0	0.025	0.35	0.60
Plasma Cholinesterase			
2471	2428	1007** (59)	724** (71)
Erythrocyte Cholinesterase			
6066	6366	5574 (8)	5558 (8)
Brain cholinesterase			
11612	11140	6594** (43)	5208** (55)

a These data were extracted from study report Table 11, page 164; n=10 rats/sex/dose.
b 8 rats/dose
c 9 rats/dose
d (Percent decrease from control value)
* or ** Significantly different from controls at $p \leq 0.05$ or 0.01.

Table 3b. Mean values of plasma and erythrocyte cholinesterase activities in the main study rats dosed with mevinphos at 0.025, 0.35, 0.60, or 0.70 mg/kg/day for up to 18 months. [a]

	Group Mean Values			
Interval (months)	3	6	12	18
Males				
Dose (mg/kg/day)	Plasma Cholinesterase			
0	471	505	678[b]	747[b]
0.025	456	538	649[b]	792[b]
0.35	279** (41)♪	291** (43)	360** (47)	365*[c] (51)
0.70	233** (51)	269** (47)	308**[d] (55)	289**[b] (61)
Dose (mg/kg/day)	Erythrocyte Cholinesterase			
0	8056	9098	6855[b]	8183[b]
0.025	8060	8982	6570[b]	8040[b]
0.35	7180** (11)	8516** (6)	5836 (15)	7552[c] (8)
0.70	7642 (5)	8284**(9)	6000[d] (12)	7195*[b] (12)
Females				
Dose (mg/kg/day)	Plasma Cholinesterase			
0	2602	2711	2197	2286[c]
0.025	2572	2716	2484	2052
0.35	1294** (50)	1230** (55)	1076** (51)	754*[e] (67)
0.60	882** (66)	919** (66)	752**[d] (66)	655**[d] (71)
Dose (mg/kg/day)	Erythrocyte Cholinesterase			
0	9300	9018	7762	8664[c]
0.025	9172	8360	7782	8008** (8)
0.35	8098 (13)	7506 (17)	7048 (9)	7510**[e] (13)
0.60	7746* (17)	7182** (20)	7251[d] (7)	7273**[d] (16)

a These data were extracted from study report, Tables 9 through 12, pages 161-165; n=10 rats/sex/dose, unless otherwise noted. Because of the low number of surviving animals (2-3 in each group) at 24 months, data from that interval are not presented. b= 8 rats/dose; c= 5 rats/dose; d= 9 rats/dose; e= 6 rats/dose♪ = (Percentage decrease from control value)

* or ** Significantly different from controls at $p \leq 0.05$ or 0.01.

F. Urinalysis: No treatment related effects were observed after 6, 12, 18, or 24 months of treatment with mevinphos.

G.. Sacrifice and pathology:

1. Organ weights -There were no dose related differences observed in absolute or relative organ weights between the treated rats and their respective control groups throughout the study.

2. Gross pathology - All gross necropsy findings detected in the treated animals were considered to be incidental. All findings are common to aging rats of this strain and were similar in frequency in controls and treated rats. The most frequent finding was pituitary gland enlargement, discoloration, and/or vascularization.

3. Microscopic pathology at necropsy:

a) Non-neoplastic: There were no treatment related non-neoplastic lesions detected at the interim or terminal sacrifices. At 104 weeks, there was an increased incidence of chronic, unilateral keratitis detected in the high-dose males (10/68 treated vs 5/67 controls), but the pathologist suggested that the finding was due to trauma and not to mevinphos toxicity. An increased incidence of hepatocellular focal vacuolation was detected in the high-dose females (15/67 treated vs 7/70 controls), but these differences were not statistically significant or dose related.

b) Neoplastic: There were no neoplasms observed at the interim sacrifice. In the terminal sacrifice animals and in those animals sacrificed moribund or found dead an increased incidence of hepatocellular adenomas was observed in the high-dose males (2.9% treated vs 0% controls). See Table 3. The study report states that the combined statistical analysis of fatal and incidental liver adenomas resulted in a significant difference between groups at **p=0.05** with a dose response significance at **p=0.06.** However, the incidence of hepatocellular adenomas in high-dose males (2.9%) was within laboratory historical control ranges (0-5%). Historical control data were presented for Sprague-Dawley rats from 6 studies conducted from 1989-1993. For males, in 314 livers examined, there was a total of 5 (1.6%) adenomas and 4 (1.3%) carcinomas. The incidence rate for the studies ranged from 0 to 5.0% for adenomas and 0 to 3.3% for carcinomas.

In high-dose females, hepatocellular adenomas were observed (3/67, 4.5% treated vs 1/70, 1.4% controls) as well as hepatocellular carcinomas (1/67, 1.5% treated vs 0/70, 0% controls). The study report states that the combined statistical analysis of fatal and incidental liver adenomas resulted in a significant difference between groups at **p=0.07** with a dose response significance at p=0.04. The combined incidence of

adenomas and carcinomas was statistically significant at p=0.02 with a dose response significance at p=0.01. The incidence of hepatocellular adenomas in high-dose females (4.5%) was not within laboratory historical control ranges (0-2%). In the historical control data for females, in 312 livers examined, there were 3 (1.0%) adenomas and 0 carcinomas. The incidence rate for adenomas ranged from 0 to 2%. However, the study report states that it was within the published historical ranges (1.4-21.7%; Mc Martin, et al; 1992; Toxicologic Pathology, 20 #2, pg 212-225). According to the study report, data were presented on 9 groups of 60 to 70 animals/sex/group for a total of 585/sex. The incidence rate for adenomas in males was 0 to 16.7% (mean 4.6%). For females, the incidence range was 1.4 to 21.7% (mean 5.8%) for adenomas. A copy of the article was not submitted.

Table 3. Incidence of hepatocellular adenomas and carcinomas in rats dosed with mevinphos at 0.025, 0.35, 0.60 (females) or 0.70 (males) mg/kg/day for approximately 24 months.[a]

Dose (mg/kg/day)	0	0.025	0.35	0.60/0.70	Historical Controls
Males					
Number examined	67	67	69	68	314
Adenoma, Hepatocellular	0 trend p=0.06	1	1	2[b] p=0.05	5 range: 0-3
% incidence	0	1.5	1.4	2.9	1.6 range:0-5
Carcinoma, Hepatocellular	4	4	2[c]	0	4 range: 0-2
% incidence	6	6	2.9	0	1.3 range: 0-3.3
Total hepatocellular tumors	4	5	3	2	9
% incidence	6	7.5	4.3	2.9	2.9
Females					
Number examined	70	66	67	67	312
Adenoma, Hepatocellular	1[d] trend p=0.04	0	0	3 p=0.07	3 range: 0-1
% incidence	1.4	0	0	4.5	1.0 range: 0-2
Carcinoma, Hepatocellular	0	0	0	1	0
% incidence	0	0	0	1.5	0
Total hepatocellular tumors	1 trend p=0.01	0	0	4 p=0.02	3
% incidence	1.4	0	0	6	1.0

a These data were extracted from the study report, page 2038, and Table 5, pages 2099 and 2100.
b First adenoma seen in males on Study Day 596
c First carcinoma seen in males on Study Day 448
d First adenomas seen in females on Study Day 636
e First carcinoma seen in females on Study Day 733

III. DISCUSSION

A. Investigators conclusions -Treatment with mevinphos for two years at dose levels of 0.60-0.70 mg/kg/day showed no evidence of oncogenicity. There were reductions in plasma, erythrocyte, and brain cholinesterase activity and accompanying clinical signs throughout the study. No other treatment related effects were observed.

The chronic LOAEL is 0.35 mg/kg/day and the chronic NOAEL is 0.025 mg/day.

B. Reviewer's discussion/conclusions -In this combined chronic/oncogenicity study, mevinphos (85.74% a.i.) was administered orally by gavage 5 days/week to a total of 80 Sprague-Dawley rats/sex/group) at nominal dose levels of 0, 0.025, 0.35, or 0.70 mg/kg/day for approximately 104 weeks. The high-dose females received 0.70 mg/kg/day until day 82 of the study; on day 83 the dose was lowered to 0.60 mg/kg/day. Ten (10) rats/sex/dose each were designated for cholinesterase determination, hematology, and clinical chemistry analyses, and 10 rats/sex/dose were terminated at approximately 52 weeks. The animals that provided blood samples were terminated at approximately 104 weeks. The carcasses of rats specified for cholinesterase measurements were discarded without pathological examination. The analytical data indicated that the variance between nominal and actual dosage to the animals was acceptable.

There were no treatment-related effects noted in food consumption, mean body weights or body weight gains. Palpable masses were observed at a similar incidence in all groups, including the controls. Hematologic, clinical chemistry, urinalysis, and ophthalmological parameters, as well as organ weights, and gross pathology were also similar in the treated and control groups.

The general condition, behavior, and appearance of treated animals at ≥6 hours post-dosing was unaffected by treatment. It was stated that the female high-dose level was reduced from 0.70 mg/kg/day to 0.60 mg/kg/day on day 83 due to signs of acute toxicity; however, the reviewers were unable to find the acute toxicity data that led to this conclusion. On days 77 through 84 the survival rates for all rats (80/sex/dose) were 99-100% and the summary tables for clinical signs did not indicate increased incidences of acute toxicity.

On days 681, 688, and 695, observations were performed 30 minutes to 6 hours post-dosing. Tremors was the most frequent finding. An increased incidence of tremors was observed in the high-dose males (14-17/24-28 treated vs 0/26 controls) and females (7-11/23-25 treated vs 0/22-25 controls) 30 minutes after dosing; 2-4 high-dose males, and 1 high-dose female were still experiencing tremors 6 hours post-dosing. Tremors were also displayed by the 0.35 mg/kg/day males and females at 30 minutes post-dosing (1-3/28-30 treated). Other signs of cholinesterase inhibition, such as exophthalmus, oral discharge, and/or anogenital staining were also observed in the treated groups, more frequently in the high-dose males. These findings all decreased over the 6 hour period.

There is some confusion about the reporting and calculation of survival rates. The sponsor stated that male and female groups showed adequate survivorship at their scheduled termination. The study report gives the following number of male survivors (%) for the increasing doses: 27/80 (34%), 31/80 (39%), 30/80 (38%) and 22/80 (28%). For females, the corresponding data are 25/80 (31%), 27/80 (34%), 29/80 (36%) and 28/80 (35%). The numerator for the survivor rates should be the number of animals at the terminal sacrifice. The denominator should be the total number of animals minus the interim and cholinesterase sacrifices. Therefore, the survival rates at the terminal sacrifice of the 67-70 rats/dose were 14/67 (21%), 18/67 (27%), 19/67 (28%) and 10/67 (15%) in controls, low-, mid-, and high-dose groups males, respectively; survival rates were 15/70 (21%), 14/67 (21%), 16/67 (24%) and 15/67 (22%) in the corresponding females groups. In general, the survival rates at termination were less than guideline requirement (not less than 25%) for this interval. From the information provided with the ophthalmological examinations (page 194 of study report), survival rates at 96 weeks were 37-51%.

At the **interim sacrifice** (Table 3a), plasma ChE activity was significantly decreased ($p \leq 0.01$) in the high-dose males (↓57%) and females (↓71%) and in the mid-dose females (↓59%); a decrease in enzyme activity in the mid-dose males (↓38%) was not statistically significant. Decreases ($p \leq 0.05$ or ≤ 0.01) in brain ChE activity were observed in the high-dose males (↓53%) and females (↓55%) and in the mid-dose group (males, ↓27%; females, ↓43%). Erythrocyte ChE was decreased only 6-8% (p=not significant) in the mid- and high-dose groups at this interval.

For the **main study groups**, ChE data at the 3, 6, 12, and 18 months were used for comparison. Because the 10 rats/sex/dose chosen for ChE activity were not replaced if they died, at 24 months, there were no surviving control females, only 3-4 surviving male controls, and only 2-3 rats/dose group. The 24 month brain ChE data were not used for comparisons. Plasma ChE activity in the main study high-dose animals was decreased ($p \leq 0.05$ or 0.01) at the 3 ,6, 12, and 18 month intervals (males, ↓47-61%; females, ↓66-71%) (Table 3b). In the mid-dose males and females, plasma ChE was statistically significantly decreased 41-51% and 50-67%, respectively. Erythrocyte (RBC) ChE activity was also decreased (↓5-20%) at these intervals in the high-dose animals; statistically significant ($p \leq 0.05$ or ≤ 0.01) decreases were noted in the high-dose males at 6 months (↓9%) and 18 months (↓12%) and in the high-dose females at 3 (↓17%), 6 (↓20%), and 18 months (↓16%). In the mid-dose males, RBC ChE was statistically significantly decreased 6-11% at the 3 and 6 month measurements and 8-15% (not statistically significant) at the 12 and 18 month measurements. The only statistically significant difference in RBC ChE in females was at the 18 month measurement (13%), although the decreases at the other time points were 9-17%. There were no treatment-related changes in ChE in the 0.025 mg/kg/day males or females, except for a statistically significant decrease in RBC ChE at the 18 month time period in the 0.025 mg/kg/day females. However this effect was not considered toxicologically significant due to the magnitude of the change, i.e. a 8% decrease. In general, the changes in RBC ChE were not considered toxicologically significant due to the magnitude of the differences between the treated and control groups.

There were no treatment related non-neoplastic lesions detected in the animals at any interval. There were no neoplasms observed at the interim sacrifice. At the scheduled terminal sacrifice and in those animals sacrificed moribund or found dead an increased incidence of hepatocellular adenomas was observed in the high-dose males (2.9% treated vs 0% controls). The statistical analysis of all liver adenomas resulted in a significant difference between groups at p=0.05 with a dose response significance at p=0.06. However, the incidence of hepatocellular adenomas in high-dose males (2.9%) was within laboratory historical control ranges (0-5%). In high-dose females, hepatocellular adenomas were observed (4.5% treated vs 1.4% controls) as well as hepatocellular carcinomas (1.5% treated vs 0% controls). The statistical analysis of all liver adenomas resulted in a significant difference between groups at p=0.07 with a dose response significance at p=0.04. The incidence of hepatocellular adenomas in high-dose females (4.5%) was not within laboratory historical control ranges (0-2%), but was within the published historical ranges cited by the Sponsor (1.4-21.7%; Mc Martin, et al; 1992; Toxicologic Pathology, 20 #2, pages 212-225).There were no carcinomas reported in females in the historical control data. The combined incidence of adenomas and carcinomas was statistically significant at p=0.02 (4/67 vs 1/70 in control; 4% vs 1.0%) with a dose response significance at p=0.01.

The study uses p values greater than the generally accepted p≤0.05 for statistical significance. Using this level, the following neoplastic findings may be biologically relevant:

Adenomas - HDT males - pairwise (p=0.05)
HDT females - trend (p=0.04)
Combined Adenomas/Carcinomas - HDT females - trend (p=0.01) and pairwise (p=0.02)

The following equivocal adverse effects were noted in the high-dose females: (i) increased alkaline phosphatase activity observed at 18 months (↑73%, p≤0.05) and 24 months (↑61%, p=not statistically significant); (ii) the increased incidence of hepatocellular focal vacuolation (15/67 treated vs 7/70 controls) detected at the terminal sacrifice; and (iii) increased incidence of hepatocellular adenomas (4.5%; significant difference between groups at p=0.07 with a dose response significance at p=0.04 for the combined statistical analysis of liver adenomas). These data suggest the liver as a possible target organ of mevinphos chronic toxicity in female rats.

The chronic LOAEL is 0.35 mg/kg/day in males and females) based on decreased plasma and brain cholinesterase activity. The chronic NOAEL is 0.025 mg/kg/day in males and females.

The submitted study is classified as **acceptable/guideline** and does satisfy the guideline requirements for a chronic toxicity study (§83-1) and a carcinogenicity study (§83-2) in rats.

C. Study deficiencies - A dose rationale was not submitted, however, this deficiency does not affect the acceptability of the study.