Mevinphos **Mouse Carcinogenicity Study (OPPTS 870.4200; §83-2)**

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DATA EVALUATION REPORT

STUDY TYPE: Mouse Carcinogenicity Study (OPPTS 870.4200)

DP BARCODE: D251794
P.C. CODE: 015801

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

TEST MATERIAL (PURITY): Mevinphos (87.7% a.i.)

SYNONYMS: none

CITATION: Atkinson J. (1989) An Eighteen Month Oncogenicity Study in Mice with Mevinphos. Bio/dynamics, Inc., East Millstone, NJ. Project No. 86-3006, February 23, 1989. MRID 41016201. Unpublished

West J. and K. Roberts (1990) Supplement to An Eighteen Month Oncogenicity Study in Mice with Mevinphos (Supplements MRID 41016201). Jellinek, Schwartz, Connolly & Freshman, Inc., Washington, D.C. Project ID JCODE 1562.1, August 31, 1990. MRID 41636801. Unpublished.

SPONSOR: AMVAC Chemical Company

EXECUTIVE SUMMARY:
In a mouse carcinogenicity study (MRIDs 41016201and 41636801), 50 CD-1 mice/sex/group were treated with mevinphos (66.5% alpha isomer and 21.2% beta isomer) in the diet for 18 months at doses of 0, 1.0, 10.0 or 25 ppm (males: 0.1, 1.5 and 3.7 mg/kg/day; females: 0.1, 1.9 and 4.8 mg/kg/day).There were no treatment-related effects, except for statistically significant decreases, although minimal (≤ 7% decrease) in body weight at the beginning of the study. Body weight gain was also decreased for the first few weeks of the study. **The NOAEL is 10.0 ppm (males: 1.5 mg/kg/day; females: 1.9 mg/kg/day); the LOAEL is 25.0 ppm (males: 3.7 mg/kg/day; females: 4.8 mg/kg/day) based on minimal body weight/body weight gain decreases at the beginning of the study.**

There was no increased incidence of neoplasms in the treated groups. Although there were minimal effects in this study, there is evidence from the range-finding study that the doses were adequate to test the carcinogenic potential of the chemical. Dose selection was based on cholinesterase changes in a three month range-finding study using doses of 0, 0.5, 1.0, 2.0 or

10.0 ppm. At termination, plasma cholinesterase levels were decreased 26 and 62% in the 2.0 and 10.0 ppm group males and 46% in the 10 ppm group females. Brain cholinesterase was decreased 16 and 22%, respectively, in the 10 ppm group males and females. There were no other treatment-related changes in the study. Cholinesterase levels were not measured in the carcinogenicity study, however it can be assumed that plasma and brain cholinesterase would have been signficantly affected at both the 10.0 and 25.0 ppm doses.

The study is classified **Acceptable (Guideline)** and **satisfies** the requirements for a mouse carcinogenicity study (OPPTS 870.4200; §83-2)

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

BACKGROUND

In a HED memo dated May 2, 1989, a cursory review of this mouse carcinogenicity study (MRID 41016201) noted that data were missing from the study report, including information on mortality, test article certification, revised histopathology tables, range-finding data and a copy of the Quality Assurance statement. The supplement, MRID 41636801, supplied this information which will be included with the review of the study.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Mevinphos
   Description: Clear to yellow liquid
   Lot/Batch #: Container 1: 50543; Container 2: No lot number;
   Containers 3 & 4: 50826
   Purity: Container 1: 62.5%; Container 2: 78.0%; Containers 3 & 4: 100%*
   Storage conditions: Refrigerated
   CAS #:

* The EPA review questioned the differences in the purity of the active ingredients of the three lots. The supplement, MRID 41636801, states that Container 1 was not used for testing. Container 2 was used as an analytical standard. Containers 3 & 4, lot number 50826, were erroneously reported as having 100% active ingredients. A letter from AMVAC Chemical Corporation states that the retained lot was reanalyzed on October 15, 1987, and found to contain 66.5% alpha isomer and 21.2% beta isomer of mevinphos for a total of 87.7% a.i.

2. Vehicle and/or positive control: None

3. Test animals:

   Species: Mice
   Strain: CD-1
   Age and weight at study initiation: 46 days old; males: 25-33 g; females: 19-25 g
   Source: Charles River Breeding Laboratories, Inc., Kingston, NY
   Housing: Animals were doubly housed during the first week of the acclimation period and individually thereafter
   Diet: Purina Certified Rodent Chow # 5002 ad libitum
   Water: automated watering system; available ad libitum
   Environmental conditions: Temperature: 66 - 76°F
   Humidity: 20 - 82%
   Air changes: Not stated

Photoperiod: 12 hour light/dark cycle
Acclimation period: 18 days

B. STUDY DESIGN:

1. In life dates -
Start of Treatment: September 22, 1986
Necropsy: Final day of necropsy was March 30, 1988

2. Animal Assignment: Animals were assigned to dose groups using a computerized random sort program as indicated in Table 1.

Table 1: Animal Assignment

		Number of Animals						
Group	Test Substance	Dose Level (ppm)	Initial	Blood Smears		Necropsy	Histopathology	Selected Histopathology
				Mo. 12	Mo. 18	Termination		
			M/F	M/F	M/F	M/F	M/F	M/F
I	Control	0	50/50	10/10	10/10	27/30	50/50	-
II	Mevinphos	1	50/50	10/10	10/10	20/30	-	50/50
III	Mevinphos	10	50/50	10/10	10/10	27/32	-	50/50
IV	Mevinphos	25	50/50	10/10	10/10	24/30	50/50	-

3. Dose Selection: The original study report stated that the doses for the study were selected at a meeting with EPA on June 23, 1986 and based on the results of a 3 month range-finding study. In the 3 month range-finding study, CD-1 mice were treated with 0, 0.5, 1.0, 2.0 or 10.0 ppm mevinphos in the diet. Lot number 5086 (66.5% alpha isomer and 21.2% beta isomer) was also used in this study. No analyses to determine the homogeneity, stability and concentration of the chemical in the diet were reported. There were no treatment-related effects, except for decreases in ChE levels. During Week 7, plasma ChE values were statistically significantly decreased 49 and 37%, respectively, for the 2.0 and 10.0 ppm group males. The plasma ChE levels were 13% decreased in the 1.0 ppm group males (not statistically significant). Plasma cholinesterase was decreased 37 and 41%, respectively, in the 2.0 and 10.0 ppm females. At termination, plasma ChE was statistically significantly decreased 26 and 62%, respectively, in the 2.0 and 10.0 ppm

group males. In females, the 10.0 ppm group females had a 46% decrease (statistically significant). Brain ChE levels were statistically significantly decreased 16 and 22%, respectively, in the 10 ppm group males and females.

In the supplement, MRID 41636801, an unaudited draft of a 4 week study was also submitted. In the 4 week study, which was originally intended as a 3 month range-finding study, there was an early termination due to possible formulation problems with the test material. No other information on the stability problems is provided in this abbreviated report. Animals were treated with either 0, 2.5, 5, 25 or 50 ppm of mevinphos in the diet. There were no treatment-related changes in mortality, body weight, food consumption and gross pathology; there were decreases in ChE levels. At study termination, plasma ChE was decreased 13-76% (all statistically significant) in treated males and 11-66% in treated females (all statistically significant, except for the low dose). Whole blood ChE was statistically significantly decreased 7-45% in treated males and 13-51% in treated females. Brain ChE was statistically significantly decreased 58-71% in the 25 and 50 ppm group males and 7-68% in the treated females. There were no RBC ChE changes from control values.

4. Dosing preparation and analysis

The study report states that appropriate amounts of test substance were mixed with standard laboratory diet once weekly and frozen due to poor stability. Diets were presented to animals three times per week. Prior to the initiation of treatment, samples were taken from the top, middle and bottom of each dose level diet for homogeneity and stability analyses. The stability of the chemical in the diet was determined on Days 1, 4, 7 and 14. Analyses of weekly samples were performed once weekly during the first four weeks of the study, at 8 and 13 weeks and then monthly throughout the study for concentration of the test material.

Results -

In the supplement, MRID 41636801, there is a September 23, 1986 letter from the study author stating that prestudy homogeneity and stability data indicated a loss of material during mixing. Although the diets were homogeneous, only 75% of the intended dose level was present. In conversation with EPA personnel, it was decided that dietary mixes would start with 30% more mevinphos than needed to yield the appropriate dose levels. Results of the analyses in the study report (Appendix H) show that the feed mixture was not stable at room temperature. After 4 days, only 75.8% of the 1 ppm dose and 63.6% of the 25 ppm dose remained. The mixture was stable when frozen. After 7 days, 99.6% of the 1 ppm dose and 88.4% of the 25 ppm dose remained. When diets were mixed once weekly and frozen, the average results of the

periodic dietary mixture analyses were as follows:

Group II (1 ppm) - 1.03 ppm ± 0.185 ppm (R.S.D. 18.0%)
Group III (10 ppm) - 10.1 ppm ± 1.60 ppm (R.S.D. 15.8%)
Group IV (25 ppm) - 25.6 ppm ± 3.98 ppm (R.S.D. 15.5 %)

5. Animals received fresh diet three times per week.

6. Statistics - Body weight, food consumption, terminal organ and body weights and organ/body weight ratios were analyzed statistically. Statistical evaluation of equality of means was made using the appropriate one way analysis of variance. Bartlett's test was first used to determine if groups had equal variance. If the variances were equal, parametric measures were used; if not, nonparametric procedures were used. The parametric procedure was the one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to dermine which means were significantly different from the control. If a nonparametric procedure was needed, Kruskal-Wallis test was used. If differences were indicated, a summed rank test (Dunn) was used to determine which treatments differed from control.

C. METHODS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality.

2. Body weight:

Animals were weighed twice pretest, weekly through 14 weeks, biweekly 16 through 26 weeks, monthly thereafter and terminally.

3. Food consumption and compound intake

Food consumption was measured pretest, weekly through 14 weeks, biweekly 16 through 26 weeks and monthly thereafter. Test substance intake was calculated from food consumption data and based on nominal concentrations.

4. Ophthalmoscopic examination

The eyes were not examined ophthalmoscopically.

5. Blood was collected via venipuncture of the orbital sinus under light ether anesthesia.

If blood was collected from an animal to be sacrificed, it was taken from the abdominal aorta. Ten animals/sex/group were selected randomly; the same animals were used at all intervals, if feasible. The CHECKED (X) parameters were examined.

a. Hematology

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

* Minimum required for carcinogenicity studies (only on Cont. and HDT) unless effects are observed based on Subdivision F Guidelines.

b. Clinical Chemistry

Clinical chemistry determinations are not required by the carcinogenicity study guidelines.

6. Urinalysis

Urinalysis determinations are not required by the carcinogenicity study guidelines.

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

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

* Required for carcinogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight reguired for non-rodent studies.

II. **RESULTS**:

A. Observations

1. Toxicity - There were no treatment-related clinical signs of toxicity.

2. Mortality - The number of animals dying, sacrificed moribund, accidently dying or missing are presented in Table 2 below. The initial cursory review noted that the mortality appeared to be excessive. The supplement, MRID 41636801, contains a letter dated July 12, 1990, from the study director that provides the historical control data from the study laboratory. The letter states that, over the past five years, the average male survivorship in chronic mouse studies is 51.6% (range: 24-78%), which is comparable to the 48.5% in this study (range: 40-54%).

Table 2: Mortality

	Males			Females		
Dose Levels (ppm)	Total F & M	Total A	Total All	Total F & M	Total A	Total All
0	23	0	23	17	3	20
1.0	29	1	30	18	2	20
10.0	23	1	24	18	0	18
25.0	26	0	26	18	2	20

Total F &M = Total animals dying spontaneously or sacrificed in a moribund condition.
Total A = Total animals missing or dying accidently.
Total All = Total of all unscheduled deaths (sum of Total SD & M and Total A)

B. Body weight

The study report states that mean body weights were decreased in the 25 ppm group females (through week 12) and males (through week 16). After this, body weight and body weight gain were comparable. In reviewing the data, the body weight changes are minimal (≤ 7% decrease), although statistically significant. When body weight gain changes are calculated, it appears that the largest effect occurred at week 1 for both the 25.0 ppm group males and females. After that, values were comparable to controls, except overall body weight gain was decreased (13%) in the 25.0 ppm males. Data are presented in Table 3.

Table 3: Body Weight and Body Weight Gain[a]

	Dose Levels (ppm)							
	Males				Females			
	0	1.0	10.0	25.0	0	1.0	10.0	25.0
Body Weight (gms)								
Pretreatment	29.0	29.4	29.3	29.1	22.5	22.0	22.3	22.1
Week 1	30.1	29.1*	29.3	28.7** (5%)	23.9	23.4	22.8** (5%)	22.5** (6%)
Week 13	37.8	36.4	37.1	35.7** (6%)	29.1	28.5	29.7	28.6
Week 26	39.6	39.5	39.6	38.9	32.1	31.4	33.0	31.6
Week 79	40.6	42.3	40.0	39.2	36.7	34.8	36.5	35.0
Body Weight Gain (gms)[b]								
Pretreatment - Week 1	1.1	-0.3	0	-0.4	1.4	1.4	0.5 (64%)	0.4 (71%)
Pretreatment - Week 13	8.8	7.0	7.8	6.6 (75%)	6.6	6.5	7.4	6.5 (2%)
Week 1-13	7.7	7.3	7.8	7.0 (9%)	5.2	5.1	6.9	6.1
Pretreatment - Week 26	10.6	10.1	10.3	9.8 (8%)	9.6	9.4	10.7	9.5
Pretreatment - Week 79	11.6	12.9	10.7	10.1 (13%)	14.2	12.8	14.2	12.9 (9%)

a Extracted from Appendix D (pages 68-77) of the study report.
b Calculated by the reviewer.
(Percentage decrease from the control value)
* $p \leq 0.05$
** $p \leq 0.01$

C. Food consumption and compound intake

1. Food consumption - There was no consistent pattern of food consumption changes in the treated groups as compared to the control. All treated male and female groups had increase food consumption at week 1, when body weight effects were most severe.

2. Compound consumption (time-weighted average) - Based on food consumption and nominal dose levels, the average test substance intake (mg/kg/day) is shown in the following table:

Dose Level (ppm)	Males	Females
Control	0.0	0.0
1.0	0.1	0.1
10.0	1.5	1.9
25.0	3.7	4.8

Extracted from text table on page 21 of the study report.

3. Food efficiency was not calculated.

D. Ophthalmoscopic examination is not required in a mouse carcinogenicity study.

E. Blood work:

Hematology - The study report states that females in the 10 and 25 ppm groups tended to have lower WBC counts at 18 months but the toxicological significance of the finding is not clear. The data are presented in Table 4.

Table 4: Mean WBC Counts at Month 18

	Dose Levels (ppm)							
	Males				Females			
WBC (thous/μL)	10.6	9.3	9.5	10.1	7.1	9.7	5.8	5.3

F. Urinalysis is not required in a carcinogenicity study.

G. Sacrifice and Pathology:

1. Organ weight - There were no treatment-related effects on organ weights.

2. Gross pathology - There were no treatment-related effects on gross pathology.

3. Microscopic pathology -

a) Non-neoplastic - Summary incidence tables were supplied in the Supplement, MRID 41636801. There was no increased incidence of non-neoplastic changes in the treated groups.

b) Neoplastic - The Supplement contained summary tables on neoplastic lesions but they were grouped using the following categories: total primary neoplasms, total benign neoplasms, total malignant neoplasms, total metastatic neoplasms, total locally invasive neoplasms and total other neoplasms. A summary of neoplasms by organ systems would have been more helpful in reviewing the data. However, review of the Incidence Summary of All Microscopic Lesions (Appendix G), showed no increased incidence of any neoplasms.

III. DISCUSSION

A. Study Author's Conclusion -

The study author concluded that the administration of doses of mevinphos up to 25 ppm for 18 months does not have a carcinogenic effect in mice. The transient body weight effect at 25 ppm seen early in the study is considered treatment-related and evidence that an MTD was approached.

B. Reviewer's Discussion and Conclusion -

In a mouse carcinogenicity study (MRIDs 41016201and 41636801), 50 CD-1 mice/sex/group were treated with mevinphos (66.5% alpha isomer and 21.2% beta isomer) in the diet for 18 months at doses of 0, 1.0, 10.0 or 25 ppm (males: 0.1, 1.5 and 3.7 mg/kg/day; females: 0.1, 1.9 and 4.8 mg/kg/day).There were no treatment-related effects, except for statistically significant decreases, although minimal (≤ 7% decrease) in body weight at the beginning of the study. Body weight gain was also decreased for the first few weeks of the study. **The NOAEL is 10.0 ppm (males: 1.5 mg/kg/day; females: 1.9 mg/kg/day); the LOAEL is 25.0 ppm (males: 3.7 mg/kg/day; females: 4.8 mg/kg/day) based on minimal body weight/body weight gain decreases at the beginning of the study.**

There was no increased incidence of neoplasms in the treated groups. Although there were minimal effects in this study, there is evidence from the range-finding study that the doses were adequate to test the carcinogenic potential of the chemical. Dose selection was based on cholinesterase changes in a three month range-finding study using doses of 0, 0.5, 1.0, 2.0 or 10.0 ppm. At termination, plasma cholinesterase levels were decreased 26 and 62% in the 2.0 and 10.0 ppm group males and 46% in the 10 ppm group females. Brain cholinesterase was decreased 16 and 22%, respectively, in the 10 ppm group males and females. There were no other treatment-related changes in the study. Cholinesterase levels

were not measured in the carcinogenicity study, however it can be assumed that plasma and brain cholinesterase would have been signficantly affected at both the 10.0 and 25.0 ppm doses.

C. Study deficiencies: None