UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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SEP 10

SEP 10 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Azinphos-methyl - Review of a Mouse Oncogenicity Study Provided in Response to a DCI

Caswell No. 374
Shaughnessy No. 058001
HED Project No. 0-0468
MRID No. 460091-01

FROM: Elizabeth A. Doyle, Ph.D. E.A. Doyle 8/29/90
Review Section I, Tox Branch II (HFAS) (H7509C)

TO: B. Lowery, PM74
Reregistration Division (H7508C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head Y.M. Ioannou 8/30/90
Review Section I, Tox Branch II (HFAS) (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief M. van Gemert 8/30/90
Tox Branch II (HFAS)
Health Effects Division (H7509C)

Registrant: Mobay Chemical Corporation

Action Requested: Review of an Oncogenicity Study in Mice submitted by the registrant in response to a data call-in.

Results of Review: Azinphos-methyl did not cause increased tumor incidence or reduced time to occurrence in male or female mice at dietary concentrations up to 40 ppm for 104 weeks. Noteworthy erythrocyte and brain cholinesterase inhibition occurred at the lowest dose level (5 ppm). An MTD was achieved in this study based upon greater than 20% inhibition of plasma, erythrocyte and brain cholinesterases at 20 and 40 ppm in diet. This study was classified as "Minimum" because of the introduction of new mice to replace any that died during the first month of the study.

Systemic NOEL < 5 ppm
Systemic LOEL = 5 ppm based on erythrocyte and brain cholinesterase inhibition

Reviewed by: Elizabeth A. Doyle, Ph.D. E.A. Doyle 8/28/90
Section I, Tox. Branch II (HFAS) (H7509C)
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Section I, Tox. Branch II (HFAS) (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: Mouse Oncogenicity (83-2b) TOX. CHEM. NO.: 374

MRID NO.: 460091-01

TEST MATERIAL: Azinphos-Methyl

SYNONYMS: Guthion; O,O-Dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] phosphorodithioate; CAS 86-50-0

STUDY NUMBER: 80-271-02

SPONSOR: Mobay Chemical Corporation
Environmental Health Research
Corporate Toxicology Department
17745 South Metcalf
Stillwell, Kansas 66085

TESTING FACILITY: Mobay Chemical Corporation
Environmental Health Research
Corporate Toxicology Department
17745 South Metcalf
Stillwell, Kansas 66085

TITLE OF REPORT: Oncogenicity Study of Azinphos-Methyl (Guthion) in Mice

AUTHOR(S): R. H. Hayes

REPORT ISSUED: April 10, 1985

CONCLUSIONS: Treatment of mice with azinphos-methyl at dietary concentrations up to 40 ppm produced no increase in tumors or treatment related adverse effects except for cholinesterase inhibition.

NOEL < 5 ppm in male and female mice
LOEL = 5 ppm in male and female mice based on inhibition of erythrocyte and brain cholinesterases.

Classification: core - Minimum
(Deficient in that a number of substitutions were made in the early stages of the study due to a failure of the registrant to adequately define appropriate doses in a preliminary study.)

This study satisfies the guideline requirements (83-2b) for an "Oncogenicity Study in Mice".

A. MATERIALS:

1. Test compound: Azinphos-Methyl, Description: Yellowish-brown flakes, Batch #79-R-225-42, Purity 88.6%, contaminants: list in Appendix 2

2. Test animals: Species: Mouse, Strain: CD_1 outbred albino, Age: 6 weeks, Weight: males - 24.0-34.2 g, females - 18.3-27.3 g, Source: Charles River Breeding Laboratories, Wilmington, Massachusetts

B. STUDY DESIGN:

1. Animal assignment - Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 104 weeks male	female	Interim Sac. - weeks male	female
1 Cont.	0	50	50		
2 Low (LDT)	5	50	50		
3 Mid (MDT)	20	50	50		
4 High (HDT)	80/40	50	50		

An additional ten mice of each sex were started on the treated diet to allow for replacements in the event of death during the first month. One month after initiation of the study, replacement mice that were not used on the study were sacrificed. The initial high dose level was 80 ppm. However, due to signs of overt toxicity after one week of feeding the treated diet, the high dose was reduced to permit continuation of the study.

2. Diet preparation - Diet was prepared weekly and stored frozen. Samples of treated food were analyzed for homogeneity, stability and concentration.

Homogeneity - Three random grab samples were taken from the top, middle and bottom of batches of 5 and 40 ppm diets. The 5 ppm diet had an average concentration of 4 ppm with a 14% range of deviation from the mean (coefficient of variation = 7%). The nominal 40 ppm diet had a mean concentration of 41.8 ppm with a range of deviation from the mean of 10% (coefficient of variation = 3%).

Stability - No decrease in test material concentration was reported in treated feed stored frozen for 21 days. The test material concentration in feed stored at room temperature for 14 days decreased by about 40%. The registrant indicated that the concentration decrease was consistent with first-order kinetics. On this basis, the half-life for the test material in feed at room temperature was estimated to be approximately 19 days.

Concentration - The concentrations of batches of diet were determined monthly. Mean concentrations for the term of the study were 4.14, 17.3 and 34.2 ppm for nominal 5, 20 and 40 ppm diets.

3. Animals received food (Ralston Purina Rodent Chow 5001-4 (Etts form)) and water ad libitum.

4. Statistics - Per the study report, "Body weight, feed consumption, hematological parameters and absolute and relative organ weights were subjected to an Analysis of Variance followed by Duncan's New Multiple Range Test. All computer-based statistical procedures utilized programs from SAS Institute, Inc., Cary, North Carolina. All significant differences were reported at the 95% confidence level."

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality except for holidays when single daily observations were made. A more thorough examination for abnormalities and masses by palpitation was made weekly.

Results - Toxicity - During the first week of the study, overt toxicity due to the test material became apparent in the high dose mice, causing the diet concentration to be reduced to 40 ppm. During this first week, four high dose females were found dead after four days and body weights were reduced by 10 and 13% in male and female mice, respectively.

No other treatment related signs of toxicity were reported.

Mortality (survival) - Thirteen mice found dead or moribund during the first month of the study were replaced. These were two control males, one control female, one 5 ppm male, one 5 ppm female, one 20 ppm female, two 80 ppm males, four 80 ppm females and one 40 ppm female.

Data in the table below summarize the mortality observed for the study period following the initial one month. No treatment related effects were reported.

Sendai virus infected the colony at about week 68 of the study. Increased mortality occurred during weeks 68 to 70 in males and females of all doses. By week 71, mortality rates had returned to preinfection levels. Mortality due to Sendai virus was similar across all treatment levels.

SUMMARY OF MORTALITY

Dietary Level (ppm)	Sex	Time (Weeks) 52 Actual	52 %	78 Actual	78 %	104 Actual	104 %
Control	Male	2	4	11	22	22	44
	Female	4	8	19	38	30	60
5	Male	0	--	9	18	17	34
	Female	3	6	9	18	20	40
20	Male	2	4	15	30	24	48
	Female	6	12	17	34	34	68
40	Male	4	8	13	26	22	44
	Female	6	12	15	30	23	46

2. Body weight - Mice were weighed weekly for the entire study period.

Results - Body weights of male and female mice fed diets containing 80 ppm of test material were lower than the controls after one week of consumption of the treated diet. The difference was statistically significant, but also reflected a difference deemed to be biologically significant as well. Following reduction of the concentration of test material in the high dose to 40 ppm, body weights for the remainder of the study were similar to the control. Although statistical significance was reported periodically during the study, the magnitude of the differences reported were small enough to represent no biologically significant difference.

Body weight gains reflected a similar pattern. During the first four-week period, the high dose males and females had notably reduced body weight gains. However, subsequent to reduction of the high dose concentration, high dose mice appeared to recover. No statistically significant differences in body weight gain were reported during the study, and no apparent biologically significant differences occurred during the remainder of the study.

At dietary concentrations ≤40 ppm, the test material had no apparent effect on body weights or body weight gains of male or female mice.

AVERAGE BODY WEIGHTS (g)

Time (Weeks)	Diet Concentration (ppm) 0	5	20	40
Males				
1	28.1	28.8	29.0*	29.3*
2	29.2	30.2*	30.6*	26.9*
3	30.9	30.9	31.4	29.2*
13	36.5	35.4	36.0	35.6
26	38.8	38.1	39.1	37.9
52	38.6	38.1	38.3	38.3
78	38.7	38.2	38.7	38.0
105	38.6	39.0	38.8	38.9
Females				
1	22.4	22.4	22.6	23.0
2	23.6	23.3	23.9	20.0*
3	24.4	23.6*	24.4	23.0*
13	28.4	27.7	28.6	29.6*
26	31.4	30.4	31.4	31.4
52	33.5	32.0*	34.0	33.8
78	35.0	33.4	35.4	34.3
105	36.4	34.4	36.5	35.3

*Significantly different from the control ($P<0.05$)

AVERAGE BODY WEIGHT GAINS (g)

Time (Weeks)	Diet Concentration (ppm) 0	5	20	40
Males				
1 - 4	3.3	2.8	3.3	1.5
5 - 13	5.0	3.7	3.7	4.9
14 - 26	2.4	2.7	2.8	2.3
27 - 52	0.5	1.0	0.1	0.4
53 - 78	-1.3	-1.3	-1.3	-1.0
79 - 104	-0.1	-0.0	0.4	-0.0
105 - 155	0.1	0.8	0.1	0.2
Females				
1 - 4	2.2	2.2	2.7	1.3
5 - 13	3.5	3.0	3.0	5.2
14 - 26	2.9	2.7	2.8	1.9
27 - 52	2.1	1.6	2.2	1.7
53 - 78	0.7	0.9	1.0	-1.1
78 - 104	1.3	0.9	1.2	0.8
105 - 155	0.3	0.4	0.9	0.6

3. Food consumption and compound intake - Consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data.

Results - Food consumption - No apparent treatment related trend in food consumption was reported. High dose males and females had reductions in food consumption at weeks 2 and 3 that were statistically significantly different from the control and appeared to be biologically significant as well. These data correspond to the body weight data recorded for this same period during which mice were receiving or recovering from 80 ppm diets. Although high dose males tended to have significantly lower food intake than the control more frequently than any other group, occasional periods of significantly elevated food consumption were also reported. Except for the first two measurement periods for food consumption (weeks 2 and 3), no apparent effect of the test material on food consumption was reported.

Efficiency of food utilization was not considered in this study.

AVERAGE WEEKLY FOOD CONSUMPTION (g/week)

Time (Weeks)	Diet Concentration (ppm) 0	5	20	40
Males				
2	40.6	39.7*	39.4	34.4*
3	38.1	39.1	39.5	31.7*
13	38.6	39.3	37.0	35.1*
26	33.6	33.7	39.6*	36.0*
52	41.8	42.0	40.0	38.6*
78	47.7	42.3*	38.2*	38.9*
105	43.1	40.3	39.9	38.9
Females				
2	39.4	39.4	40.2	33.4*
3	41.5	41.1	42.6	32.5*
13	41.9	39.4	41.5	40.7
26	36.0	37.5	45.3*	41.1*
52	42.4	40.7	45.4	43.4
78	53.1	45.7*	48.2*	40.6*
105	39.8	39.2	44.7	40.1

*Significantly different from the control ($P<0.05$)

Compound intake - Values for intake as mg of test material/kg/day were consistent with expected levels based upon the study data. For males, compound intakes were 0.788, 3.486 and 11.330 mg/kg/day for the 5, 20 and 40 ppm dietary groups, respectively. Comparable intakes in females were 0.977, 4.116 and 14.303 mg/kg/day.

4. Ophthalmological examinations were not performed.

5. Blood was collected at 6, 12 and 24 months for hematology and cholinesterase analysis from ten animals. The CHECKED (X) hematology parameters were examined. No other clinical chemistry data were provided.

a. Hematology:

X	Hematocrit (HCT)	X	Total plasma protein (TP)
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)

Results - No consistent effects of the test material on hematological parameters were evident. Isolated statistically significant differences occurred, but no pattern could be discerned. HCT and MCHC were the most consistently perturbed parameters, but did not increase or decrease consistently in males compared to females or across different time periods.

b. Cholinesterase - Plasma cholinesterase activity was reduced relative to the control in males and females from the 20 and 40 ppm treatment groups in a dose related manner at all time periods. Although occasional reductions occurred in the 5 ppm treatment group, no consistent pattern of reduction was apparent at this dose. Erythrocyte cholinesterase activity was also reduced in a dose related manner at all sampling points in male and female mice. However, mice of both sexes from the 5 ppm group also had reduced cholinesterase relative to the control at 6 and 24 months. At the 12 month sampling point, only females had reduced erythrocyte cholinesterase activity; males were similar to the control. Brain cholinesterase was only measured at the terminal sacrifice. Brain cholinesterase was reduced in a dose related manner in males and females from all treatment groups. The 5 and 20 ppm group males had similar levels of cholinesterase, slightly reduced relative to the control, but females exhibited a clear dose response. The 40 ppm groups were reduced to approximately one third of the control activity in both sexes.

CHOLINESTERASE SUMMARY

Dose Level (ppm)	Duration of Treatment (months) 6 ♂	6 ♀	12 ♂	12 ♀	24 ♂	24 ♀
Plasma (μmol/ml/min, % of control)						
0	3.11 (100.0)	5.76 (100.0)	3.88 (100.0)	6.51 (100.0)	4.33 (100.0)	4.98 (100.0)
5	3.33 (107.1)	5.45 (94.6)	4.81 (124.0)	5.44 (83.6)	3.95 (91.2)	4.93 (101.0)
20	2.57 (82.6)	3.08 (53.5)	2.63 (67.8)	3.27 (50.2)	2.97 (68.6)	3.86 (77.5)
40	1.62 (52.1)	1.48 (25.7)	1.32 (34.0)	1.50 (23.0)	1.89 (43.6)	1.65 (33.1)
Erythrocyte (μmol/ml/min, % of control)						
0	1.33 (100.0)	1.16 (100.0)	1.04 (100.0)	0.87 (100.0)	0.95 (100.0)	0.79 (100.0)
5	1.11 (83.5)	1.03 (88.8)	0.99 (95.2)	0.81 (93.1)	0.80 (84.2)	0.62 (78.5)
20	0.88 (66.2)	0.67 (57.8)	0.45 (43.3)	0.39 (44.8)	0.54 (56.8)	0.40 (50.6)
40	0.67 (50.4)	0.63 (54.3)	0.20 (19.2)	0.20 (23.0)	0.35 (36.8)	0.32 (40.5)
Brain (μmol/g/min, % of control)						
0	-- --	-- --	-- --	-- --	14.7 (100.0)	14.4 (100.0)
5	-- --	-- --	-- --	-- --	12.9 (87.8)	13.6 (94.4)
20	-- --	-- --	-- --	-- --	12.3 (83.7)	10.6 (73.6)
40	-- --	-- --	-- --	-- --	5.4 (36.7)	4.7 (32.6)

6. Urinalysis - Not performed.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

X	X	X
Digestive system	Cardiovas./Hematol.	Neurologic
Tongue	X Aorta	XX Brain
X Salivary glands	XX Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord (3 levels)
X Stomach	X Lymph nodes	X Pituitary
X Duodenum	XX Spleen	X Eyes (optic nerve)
X Jejunum	X Thymus	Glandular
X Ileum	Urogenital	X X Adrenals
X Cecum	XX Kidneys	Lacrimal gland
X Colon	X Urinary bladder	X Mammary gland
X Rectum	XX Testes	X Parathyroids
XX Liver	Epididymides	X Thyroids
X Gall bladder	X Prostate	Other
X Pancreas	X Seminal vesicle	X Bone
Respiratory	XX Ovaries	X Skeletal muscle
X Trachea	X Uterus	X Skin
XX Lung		X All gross lesions and masses
X Larynx		

Results -

a. Organ weight - No treatment related effects on organ weights or organ weights as a percent of body weight were reported.

b. Gross pathology - No treatment related effects were reported.

c. Microscopic pathology

1) Non-neoplastic - No treatment related effects were reported.

2) Neoplastic - No treatment related effects were reported.

D. DISCUSSION: During the first week of this study, the high dose level, 80 ppm caused excessive toxic response resulting in a reduction of this dose to 40 ppm. A group of extra mice were started in parallel to the main study group, and were used as replacements for any mice found dead or moribund by the end of the first month. At that time, remaining replacement mice were sacrificed. This procedure is questionable in that it introduces potential statistical problems with respect to mortality and biological problems with respect to sensitivity of the study animals to the test material. The number of replacements required were not identical across treatment groups. Tox Branch considers this to be a flaw in the study, but has accepted the study as usable with respect to the endpoint of oncogenicity.

Subsequent to the reduction in the high dose concentration, no treatment related effects were reported at any dietary concentration with the exception of cholinesterase inhibition. No increase in any tumor was reported and mortality was not affected by the test material over the term of the study. The 40 ppm was considered to represent an MTD on the basis of plasma, erythrocyte and brain cholinesterase inhibition in excess of 20%.

E. CONCLUSIONS: Treatment of mice with azinphos-methyl at dietary concentrations up to 40 ppm produced no increase in tumors or treatment related adverse effects except for cholinesterase inhibition.

NOEL < 5 ppm in male and female mice
LOEL = 5 ppm in male and female mice based on inhibition of erythrocyte and brain cholinesterases.

Classification: core - Minimum
(Deficient in that a number of substitutions were made in the early stages of the study due to a failure of the registrant to adequately define appropriate doses in a preliminary study.)

This study satisfies the guideline requirements (83-2b) for an "Oncogenicity Study in Mice".