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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Azinphos-Methyl

Project No. 0-0401
TOX Chem No.: 374

FROM: Ray Landolt *RL*
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THRU: Mike Ioannou, Acting Section Head *M. Ioannou 4-12-89*
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and

Marcia van Gemert, Acting Chief *Marcia van Gemert 4/14/89*
Toxicology Branch II - Herbicide, Fungicide, and
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Registrant: Mobay Corporation, letter of November 4, 1988

Action Requested:

Review a acute delayed neurotoxicity study (81-7)
submitted in response to the Registration Guidance Document
dated September 11, 1986.

JG

Reviewed By: Ray Landolt *RL 4/12/89*
Section I, Toxicology Branch II - HFAS (H7509C)
Secondary Reviewer: Mike Ioannou
Section I, Toxicology Branch II - HFAS (H7509C)

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DATA EVALUATION RECORD

Study: Acute Delayed Neurotoxicity Study (81-7)

FOX Chem No.: 374
MRID No.: 408831-01

Study No.: Mobay Report No. 94862

Testing Laboratory: Hazleton Laboratories, Inc.
No. HLA 6232-101

Author: Steven M. Glaza

Study Date: September 22, 1988

Sponsor: Mobay Corporation

Test Material: Azinphos-Methyl with a purity of 85% was used
in this study

Synonyms: Guthion

Study Title: Acute Delayed Neurotoxicity Study in the Domestic
Fowl.

Conclusions:

1. Classification of Data - Minimum
 - a. Deficiency - The criteria for the neuropathological grading system was not submitted with this report.
2. Azinphos-methyl was negative for neurotoxicity when administered to the laying hen at the LD₅₀ 330 mg/kg level.

A. Materials:

1. Animals - Sixty (46-week-old) White Leghorn laying hens were purchased from Pick's Feed and Grain of Spring Green, Wisconsin.
2. Test Material - A brown waxy solid identified with Reference No. 70R-225-42 and a purity of 85% was used to prepare the use concentration of 33 mg/ml in corn oil. The oral LD₅₀ 330 mg/kg in unprotected hens was determined previously (HLA Study No. 6232-100).
3. The positive control tri-ortho-tolylphosphate (TOTP) was administered at 60 mg/mL in corn oil.

B. Study Design:

<u>Group</u>	<u>Treatment</u>	<u>Dose*</u> mg/kg	<u>Number of</u> <u>Animals</u>
0	Untreated control	--	10
1	Vehicle control (corn oil)	--	10
2	Positive control (TOTP)	600	10
3	Azinphos-methyl	330	30

*Volume of 10.0 ml/g was administered by gavage based on the fasted body weights.

"All positive control and Guthion-treated animals received an intramuscular injection of atropine sulfate (15 mg/kg) approximately 15 minutes before oral gavage to protect against sudden death because of TOTP or Guthion toxicity ... Additional treatments of atropine were given to Guthion-treated hens as needed up to approximately 24 hours postdose."

All animals were fasted for approximately 16 hours prior to dosing after which food (Purina Lavena Chicken Food No. 6051) was available ad libitum. Tap water was provided ad libitum.

On day 21, because of the absence of definitive neurotoxic effects in the surviving test, vehicle control animals and in six positive control animals, a second treatment was administered to the animals based on their Day 21 body weights.

Statistical Analysis - Standard one-way analysis of variance (ANOVA) was used to analyze body weight data. Levene's test was done before ANOVA to test for variance homogeneity. The ANOVA was then done on the homogeneous or ranked data. If the ANOVA was significant, Dunnett's t-test was used for pairwise comparisons between groups. All group comparisons found to be statistically significant were reported at the 5% significance level. Histopathological lesion grades were evaluated for statistical differences between groups utilizing an ANOVA and Duncan's Multiple Range test. All statistically significant differences were reported at the 95% confidence level.

C. Method and Results:

1. Observations - "The animals were observed for mortality, clinical signs of toxicity, and ataxia based on forced locomotor activity. These animals were observed at hourly intervals for the first 4 hours after dosing and daily thereafter until sacrifice (Day 21, 43, or 44). Individual body weights were recorded on the day of dosing and on Days 7, 14, 20, 21, 28, 35, 43, and 44."

Locomotor activity was graded as follows for Grade:

1. Slight incoordination, occasional stumbling or wing drooping, especially after exertion.
 2. Staggering gait: tail and leg reflexes may be affected. When forced into flight from a low perch, the bird lands awkwardly.
 3. Continuous staggering gait. Bird rests often. Tail and leg reflexes noticeably affected.
 4. Bird stands for short period only. Normally moves by shuffling on hocks. Leg and tail reflexes noticeably affected.
 5. Bird is unable to stand. Weak limb movement. Tail and leg reflexes virtually nonexistent.
2. Negative (Untreated) Control - No mortality or signs of toxicity, i.e., ataxia, were observed during the 44-day observation period. The mean body weights recorded during the 44-day observation period were comparable to the initial pretest body weights for this group of animals.
 3. Vehicle (Corn Oil) Control - No mortality or signs of toxicity, i.e., ataxia, were observed during the 44-day observation period. However, liquid-like feces were observed within 24 hours of the initial and redosing

period, but were not apparent by the 48-hour observation. The mean body weights recorded during the 44-day observation period were comparable to the initial pretest body weights for this group of animals.

4. Positive (TOTP-600 mg/kg) Control - No mortality was observed during the 44-day observation period. Liquid-like feces were observed within 24 hours of the initial and redosing period but were not apparent by the 96-hour observation. Hypoactivity was observed during the initial and redosing periods. The birds dosed with TOTP exhibited slight incoordination: (Grade 1) and occasional staggering gait (Grade 2) during the initial 21 days and the 22-to 44-day redosing period. Four hens were sacrificed on Day 23 and six hens were terminally sacrificed on Days 45 and 46. Body weights of the TOTP group were comparable to the untreated control group during dosing the initial 21-day period. A significant ($p < 0.05$) decrease in body weight (10%) was reported on Day 28 following the repeat dose of TOTP. The body weights of these hens remained depressed (not statistically significant) for the duration of the study.
5. Azinphos-Methyl (300 mg/kg) - Within 3 to 4 days of the initial dose, 18/30 hens were found dead. Ataxia (Grade 5) prostration, hypoactivity, and liquid feces were observed among these animals and those surviving the initial dose. A significant ($p < 0.05$) decrease in body weight (17%) was reported during the 21-day period following the initial dose. Following the repeat dose on Day 22, 1/12 of the surviving hens were found dead within 2 days of the second dose. Ataxia (Grade 4 and 5), hypoactivity and liquid feces were observed among these animals. These signs of toxicity including ataxia were reversible within 21 days of the initial and repeat doses. A significant ($p < 0.05$) decrease in body weight (23%) was reported during the 21-day period following the repeat dose.
6. Gross Necropsy - Macroscopic observations revealed no abnormalities related to the administration of TOTP or azinphos-methyl.
7. Histopathological Examinations - All animals that died on test were necropsied but not perfused. Moribund animals were anesthetized and sacrificed. Four positive control animals were sacrificed on Day 21; all other animals that survived were sacrificed on Day 43 or 44. At the time of necropsy, all moribund animals and animals that survived to termination were anesthetized with pentobarbital and sacrificed using a systemic perfusion of 10%

phosphate-buffered formalin. Tissues were collected, trimmed, and processed with histologic sections prepared from the following:

- a. Brain - Sections were taken from the medulla/pons, cerebellar cortex, and cerebral cortex.
- b. Spinal Cord - Cords were left intact in the vertebra after a complete dorsal laminectomy and transection cranial to the cervical intumescence.
- c. Peripheral Nerves - Sections were taken of sciatic, fibular, and tibial nerves.

All of the hens of groups 1 and 2 were sacrificed and perfused on days 45 and 46. In the TOTP group, four hens on Day 23 and six on Days 43 and 46 were sacrificed and perfused. Eleven hens of the azinphos-methyl group were sacrificed and perfused on days 45 and 46.

Of the 18 hens that died during the study, varying degrees of postmortem autolysis within the nervous tissues were observed in the 18 hens dosed with azinphos-methyl. Neuro-pathological changes were graded on a scale of 1 to 4 in severity with grade 1 representing minimal degenerative change. A histopathological summary of the nervous tissues examined at the termination of the study from 10/10 of the untreated group, 10/10 of the vehicle control group, 6/10 of the TOTP control group, and 11/30 of the azinphos-methyl test group is presented in the following tables from the report.

A statistically significant ($p < 0.05$) increase in the incidence and/or severity of degeneration of digestive chambers, macrophage accumulation, axonal degeneration, and demyelination was reported for the TOTP-treated hens as compared to the control animals.

No statistically significant differences in the incidence or severity of lesions were reported between the controls and the azinphos-methyl treated hens.

Histopathology Summary of Nervous Tissues Examined at the Termination of the Study

No./Group Sacrificed at Termination	Degeneration Chamber			Macrophage Accumulation			Axonal Degeneration			Neuronal Degeneration			Demyelination		
	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
Brain	-	-	6	-	-	6	-	-	5	-	-	1	-	-	6
Average Grade	-	-	2.3	-	-	1.8	-	-	2.0	-	-	2.0	-	-	1.7
# Affected	0	0	6	0	0	6	0	0	5	0	0	1	0	0	6
% Affected	0	0	100	0	0	100	0	0	83	0	0	9.1	0	0	100
Cervical Cord	3	8	6	2	3	6	3	3	6	1	-	-	-	-	5
Thoracic Cord	6	6	6	2	3	4	3	-	1	3	-	-	-	-	3
Lumbar Cord	-	-	1	-	-	3	1	-	-	2	-	-	-	-	1
Average Grade	1.1	1.3	2.1	1.0	1.2	1.8	1.2	1.3	1.3	1.5	2.0	-	-	-	1.9
# Affected	6	9	6	3	6	6	6	3	4	6	1	0	0	0	5
% Affected	60	90	100	30	60	100	54.5	30	40	100	9.1	0	0	0	83
Sciatic Nerve	-	1	5	-	-	2	-	-	2	-	-	-	-	-	2
Tibial Nerve	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Fibular Nerve	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Average Grade	-	1.0	1.4	-	-	1.5	-	-	1.5	-	-	-	-	-	1.8
# Affected	0	1	5	0	0	2	0	0	2	0	0	0	0	0	2
% Affected	0	10	83	0	0	33	0	0	33	0	0	0	0	0	33

Histopathology Summary of Nervous Tissues Examined at the Termination of the Study

No./Group Sacrificed at Termination	Perivascular Cuffing			Vacuolar Degeneration			Inflammation			Post-mortem Autolysis			Lymphocyte Accumulation			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Brain	1	-	1	3	1	-	-	2	-	-	-	-	-	-	-	1
Average Grade	2.0	-	2.0	2.3	2.0	-	-	2.0	-	-	-	-	-	-	-	2.0
# Affected	1	0	1	3	1	0	0	0	0	0	0	0	0	0	0	1
% Affected	10	0	16.7	27.3	10	0	0	0	0	0	0	0	0	0	0	0.1
Cervical Cord	2	3	4	2	-	-	-	-	-	-	-	-	-	-	-	-
Thoracic Cord	1	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-
Lumbar Cord	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Average Grade	2.0	1.5	1.8	1.8	2.0	-	-	-	-	-	-	-	-	-	-	-
# Affected	3	4	4	3	1	0	0	0	0	0	0	0	0	0	0	0
% Affected	30	40	67	27.3	10	0	0	0	0	0	0	0	0	0	0	0
Sciatic Nerve	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Tibial Nerve	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Fibular Nerve	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	1
Average Grade	-	2.0	1.0	3.5	-	-	-	-	-	-	-	-	-	-	3.0	2.0
# Affected	0	1	1	2	0	0	0	0	0	0	0	0	0	0	2	1
% Affected	0	10	16.7	18.2	0	0	0	0	0	0	0	0	0	0	20	16.7