

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

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MEMORANDUMOFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. No. 100-524. Evaluation of Acute Delayed
Neurotoxicity Study in Hens with Diazinon MG-8
(Technical)

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Record No.: 234060MRID/Accession No.: 406608-06Tox. Chem. No.: 342T.B. Project No.: 9-0277

Toxicology Branch I/IRS has completed an evaluation of the
following study:

Acute Delayed Neurotoxicity of Diazinon MG-8 in Domestic
Fowl; Stillmeadow, Inc.; No. 5152-87; May 23, 1988.
EPA Guidelines No.: 81-7

Diazinon MG-8 (Two single oral doses: 28.09 mg/kg on day 0
and 13.78 mg/kg on day 21) did not cause delayed neurotoxicity in
hens (age at first dosing: 11.5 months), even though the first
dose was (erroneously) much higher than the acute oral LD₅₀ of
12.49 mg/kg. Positive results (changes in gait and locomotor
activity, and lesions in brain, spinal cord and peripheral
nerves) were observed with Tri-o-tolyl phosphate (TOCP), a known
neurotoxic agent.

Classification of Study: Core-Minimum

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DATA EVALUATION REPORT

STUDY TYPE: 81-7. Acute Delayed Neurotoxicity (Hen)

TOX. CHEM. NO.: 342

MRID NO.: 406608-06

TEST MATERIAL: Diazinon MG-8 (Technical); Purity: 87%;
Batch No.: FL872049; Stable at room
temperature (expiration date: July, 1990);
Insoluble in water; Clear, amber liquid.

STUDY NUMBER(S): 5152-87

SPONSOR: Ciba-Geigy Corporation, Agricultural Division,
Greensboro, NC

TESTING FACILITY: Stillmeadow, Inc., Houston, Texas

TITLE OF REPORT: Acute Delayed Neurotoxicity of Diazinon MG-8
in Domestic Fowl

AUTHOR(S): Lawrence J. Jenkins

REPORT ISSUED: May 23, 1988

CONCLUSIONS:

Diazinon MG-8 did not cause delayed neurotoxicity in hens under the conditions of this study. Positive results were obtained with Tri-o-tolyl phosphate (TOCP), a known neurotoxic agent.

Groups of 11.5-months old hens were treated (single doses by gastric intubation) with corn oil (Group I; vehicle control), TOCP (Group II; positive control) or Diazinon MG-8 (Group III; 28.09 mg/kg*) and observed for 3 weeks. On day 21, Groups I and III were redosed (except that the dose of Diazinon MG-8 used was 13.78 mg/kg), observed for another 21 days and sacrificed on day 42. Group II was sacrificed on day 22 without redosing. The hens were protected from acute cholinergic effects by injections of atropine sulfate and 2-PAM. Delayed neurotoxicity (changes in gait and locomotor activity) and lesions in brain, spinal cord and peripheral nerves were observed only in the positive control group.

CLASSIFICATION OF STUDY: Core-Minimum

*This dose, more than twice as high as the acute oral LD₅₀,

was administered unintentionally because of an error in the preparation of a dosing solution. The acute oral LD₅₀ for these hens was 12.49 mg/kg, in the absence of atropine sulfate and 2-PAM, and the intended dose for the delayed neurotoxicity study was about 13 mg/kg.

EXPERIMENTAL PROCEDURES:

Dosing for the acute delayed neurotoxicity study was started on January 19, 1988, and the study was terminated on March 1, 1988.

Domestic hens (Production Red Heavy Breed strain) were assigned randomly to three groups and treated (single doses by gastric intubation) as follows: Group I (10 hens: vehicle control), with corn oil, 1 ml/kg b.w.; Group II (8 hens: positive control group), with Tri-o-tolyl phosphate (TOCP), 500 mg/kg b.w.; and Group III (18 hens: test group), with Diazinon MG-8, 28.09** mg/kg b.w., administered in corn oil. The hens were fasted for at least 12 hours prior to dosing. In order to protect the hens from acute cholinergic effects of Diazinon MG-8 and TOCP, all hens (including the vehicle controls) were injected intramuscularly with atropine sulfate (10 mg/kg b.w.) 1 hour prior to dosing, with 2-PAM (50 mg/kg b.w.) immediately after dosing, and again with atropine sulfate and 2-PAM concurrently at 1 and 5 hours after dosing. Because of toxic signs, two hens in Group III required protective treatment also on day 1. Following a 21-day observation period, hens in Groups I and III were redosed, except that the dose of Diazinon MG-8 used was 13.78 mg/kg b.w. The hens were observed for another 21 days before being sacrificed on day 42. Hens in Group II (positive control) were sacrificed on day 42 without redosing.

**The dose level of Diazinon MG-8 used in the acute delayed neurotoxicity study was to be based on an acute oral LD₅₀, obtained from a study with the same strain of hens and in the absence of atropine sulfate and 2-PAM. In the acute oral toxicity study, groups of 4 hens received single doses of Diazinon MG-8 (5, 10, 12.5 or 15 mg/kg b.w.) by gavage and then were observed hourly for mortality. Using the procedure of J. T. Litchfield and F. Wilcoxon (1949; J. Pharmacol. Expt. Therap. 96, 99-115), LD₅₀ = 12.49 mg/kg b.w. with 95% confidence of 11.44-13.64 mg/kg b.w. was calculated. Based on the LD₅₀, it was decided to administer about 13 mg of Diazinon MG-8/kg of body weight to Group III hens in the acute delayed neurotoxicity study. However, because of an error in the preparation of the dosing solution, the first dose of Diazinon MG-8 administered to Group III was 28.09 mg/kg b.w. or more than twice as high as the intended target dose.

Hens used in the acute delayed neurotoxicity study and in the preliminary studies (range-finding for acute oral LD₅₀ and acute oral LD₅₀) were: 1) Obtained from Texas Animal Specialties, Humble, Texas; 2) Housed individually at 58-70° F, relative humidity of 39-68%, and 12-hour light/dark cycle; 3) Identified by leg bands; and 4) Fed unrestricted amounts of food (Purina Layena Poultry Feed) and tap water. At the initiation of the acute delayed neurotoxicity study, the hens were approximately 11.5 months old and weighed 1.6-2.2 kg.

The following parameters were examined:

1. Daily observation for toxic signs.
2. Observations for delayed neurotoxicity (changes in gait and locomotor activity). Beginning 3 days after dosing, all hens in Groups I, II and III were observed and scored independently by two different observers three times per week for signs of delayed neurotoxicity. During each observation period, hens were prodded with a pole to walk around their pens (forced motor activity) until the score of the gait could be determined.
3. Body weight, on the day of dosing and weekly thereafter.
4. Food consumption, weekly.
5. Necropsy, on all hens sacrificed at scheduled times. The nonsurvivors were not necropsied.
6. Histopathology, on all hens but the nonsurvivors. The following tissues were examined: brain (medulla oblongata), spinal cord (cervical, thoracic and lumbar regions) and peripheral nerves (sciatic and tibial). Tissue sections were stained with hematoxylin and eosin (standard stains) and also with myelin- and axon-specific stains, Luxol Fast Blue (brain and spinal cord) and Holmes silver nitrate (peripheral nerves).

Statistical Analyses

Body weights and food consumption data were evaluated by analyses of variance (ANOVA), Student's and Dunnett's t-tests, Kruskal-Wallis X²-test, and Ryan's procedure for the Mann-Whitney U-test. If p was greater than 0.05, the finding was regarded as non-significant. (See Attachment I for details).

RESULTS

Mortality

Three hens were found dead, one in Group I (vehicle control) on study day 7 and two in Group III (Diazinon-treated group) on days 5 and 21 (6 hours after redosing).. There was no mortality

in Group II (positive control).

Toxic Signs

Following the first dosing, decreased activity was observed in 3/10 hens in Group I, 5/8 in Group II and 8/18 in Group III. Decreased activity was slight in Groups I and II, and slight to extreme in Group III, and lasted for 1 to 3 days in Group I, 6 hours to 2 days in Group II, and 6 hours to 4 days in Group III. Other toxic signs observed shortly after dosing included convulsions in 1 Group I hen that did not survive the study and ataxia, salivation, respiratory gurgles and nasal discharge in 2 Group III hens (1 of which did not survive the study).

Following redosing, decreased activity (very slight to slight) occurred in 2/9 hens in Group I and 9/17 hens in Group III, and lasted in all instances for 6 hours. Other toxic signs were not observed. Group II hens were not redosed but were sacrificed on study day 22.

Delayed Neurotoxicity

Following the first dosing, delayed neurotoxicity was not observed in the vehicle control (Group I) and Diazinon-treated (Group III) hens. In Group II (positive control), 3 hens showed unsteady gait on study day 10. From day 13 through day 22, signs for all positive control hens progressed from slight unsteadiness in walking to marked staggering and occasional falling. On day 22, 4 of 8 hens were unable to walk. The mean neurotoxicity scores for the 8 hens in Group II ranged from 2.5 to 4.5 on study day 22.

Following redosing, neurotoxicity scores for all Group III hens were 0, except for one hen which had an average score of 0.5 on day 41 due to slight unsteadiness in walking. This single occurrence of unsteadiness at the termination of the study was not attributed to treatment.

Scoring for neurotoxic signs was performed according to the procedure of H. W. Chambers and J. E. Casida (1967; Toxicol. Appl. Pharmacol. 10, 105-118), as follows:

<u>Score</u>	<u>Scoring Criteria</u>
0	No detectable signs.
1	Slight unsteadiness in walking.
2	Marked staggering and occasional falling.
3	Extreme difficulty in walking and falling often.

- 4 Inability to walk; standing with difficulty.
- 5 Complete motor paralysis of legs; usually lying on side.

Body Weight

Hens in Groups I and III gained weight during the 6-week study period (2.6 and 3.2%, respectively) when the mean final body weights for each group were compared with initial weights.

With one exception, all hens in Group II lost weight during the first study week (4.7%) and gained weight during the second week (3.7%) when these body weights were compared with predosing weights (study week 0). Weight loss (7.7%) reoccurred during the third week when signs of neurotoxicity appeared. The body weight of Group II hens were also significantly smaller (10.5%; $p < 0.01$) than those of vehicle controls (Group I) during week 3, just before Group II was sacrificed.

The three hens which died during the study did not have their terminal weights recorded.

Food Consumption

No significant differences between the Diazinon-treated and vehicle control groups were noted for mean food consumption values. In the positive control group, food consumption declined by 18.3% after week 2 when neurotoxicity became apparent.

Necropsy

No abnormalities were observed in any group. The 3 nonsurviving hens, 1 in Group I and 2 in Group III, were not necropsied.

Histopathology

Hens from the vehicle control and Diazinon-treated groups had no lesions in brain, spinal cord and peripheral nerves (tissues examined). All positive control hens had some degree of degeneration and swelling of the axons. Mild to moderate lesions (mostly grades 3-5) were observed in all positive control hens with the most severe degeneration occurring in the cerebellum, peripheral nerves, and the ventral tracts of the spinal cord.

The nonsurvivors were not examined.

The lesions were graded for severity according to the procedure of A. A. Bickford and G. L. Sprague (1983; Neurotoxicology 3, 321-332), as follows:

- 1 - rare, minimal
- 3 - few, mild
- 5 - several, moderate
- 7 - numerous, extensive

COMMENTS

According to the testing laboratory, the protocol deviations (nonsurviving hens were not necropsied and the first dose of Diazinon MG-8 was inadvertently over twice as high as the target dose of 13 mg/kg b.w.) did not affect the integrity of the study. The three hens which did not survive the study (1 in the vehicle control and 2 in the Diazinon MG-8-treated group) died shortly after treatment and their deaths were therefore not due to delayed neurotoxicity. Also, sufficient numbers of hens were available in these groups to permit an assessment of the delayed neurotoxic potential of Diazinon MG-8.

The inadvertent doubling (28.09 mg/kg b.w.) of the intended dose for the Group III hens on day 0 had no impact on the study because most of the hens survived this dose and tolerated the second dose of 13.78 mg/kg b.w. Both of these doses were greater than the unprotected LD₅₀ of 12.49 mg/kg b.w.

Toxicology Branch/HED agrees with the above comments.

In summary, Diazinon MG-8 did not induce delayed neurotoxicity in the hens under the conditions of this study. Positive results were obtained with TOCP, a known neurotoxic agent.

Classification of study: Core-Minimum

Good Laboratory Practice Statement, signed and dated May 31, 1988, was included in the report.

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Attachment I

Statistical Analyses

Body weight and food consumption data were analyzed for significant differences by analyses of variance (ANOVA). Student's and Dunnett's t-tests were used to establish significance, indicated by the ANOVA, between the Vehicle Control and the remaining groups. Significant findings, as interpreted by Dunnett's t-test, are detailed in Results and Discussion as well as in the summary tables. Body weight gains were compared with the Kruskal-Wallis X^2 -test (Kirk 1982). Ryan's procedure for the Mann-Whitney U-test (Kirk 1982) was used to determine significant differences between the Vehicle Control and the remaining groups following the detection of a significant difference with the Kruskal-Wallis test. All statistical analyses were tested for significance at the $p \leq 0.05$ and $p \leq 0.01$ levels with a null hypothesis of no difference between respective means. If p was greater than 0.05, the test was judged non-significant.

Kirk, R.E. (1982). Experimental Design: Procedures for the Behavioral Sciences. Brooks/Cole Publishing Company, Belmont, California.
911 pp.