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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCESMEMORANDUM

SUBJECT: EPA Reg. No. 9618-23 - Diazinon: Review of acute delayed neurotoxicity study in hens submitted as 6(a)(2) data.

TOX CHEM. No.: 342
TOX PROJECT No.: 8-0163
Record No.: 206566

FROM: John Doherty *John Doherty* 12/14/87
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: George LaRocca
Product Manager #15
Registration Division (TS-767)

THRU: Edwin Budd
Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: Theodore Farber, Ph.D.
Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Budd
12/14/87

W2 Brown
12/14/87

The Trans Chemic Industries, Inc. has submitted under FIFRA Section 6(a)(2) an acute delayed neurotoxicity study with hens that was sponsored by the Nippon Kayaku Co., Ltd, of Japan. The registrant acknowledges (refer to letter from George E. Miller dated September 4, 1987 to George LaRocca PM #15, Reg. Div., EPA.) that the study has "possible equivocal results" which may indicate a delayed neurotoxic effect. The registrant indicated that neither the study author, the original sponsor (Nippon Kayaku Co.) nor themselves (Trans Chemic Co.) have concluded that the study clearly demonstrated that diazinon causes delayed type neurotoxicity.

The study was reviewed by Toxicology Branch (TB) and the following comments apply.

Toxicology Branch Comments

1. There were five hens out of twelve treated with diazinon which exhibited "axonal degeneration" but none of six vehicle control hens had this lesion. These histopathological observations were generally not clearly and unequivocally corroborated by behavioral signs but they were qualitatively similar but less severe than the histopathological observations noted in the hens dosed with the positive control (TOCP). The observation of "axonal degeneration" in the hens treated with diazinon provides evidence that diazinon may cause the delayed type neurotoxic response.
2. TB requests that a second acute delayed type neurotoxicity study with diazinon supplied by the Nippon Kayaku Co., Ltd. be provided to help to clarify the potential for diazinon to induce the delayed type neuropathy.

In conducting this second study, the testing laboratory is advised to include the following:

- a. at least 12 hens per dose level for all groups including the vehicle and positive control groups.
- b. three dose levels of diazinon (atropine and pralidoxime may and should be used to protect the birds from acute ChE inhibition poisoning). The three dose levels recommended are as follows:
 - i. high dose - a dose level greater than the LD₅₀
 - ii. mid dose - the LD₅₀ dose level
 - iii. low dose - one half of the LD₅₀ dose level
3. Pending receipt of the study as above, TB will decide if the available data justify determining that diazinon is considered potentially capable of inducing the delayed type neurotoxicity response and if it will be necessary to require a subchronic neurotoxicity study to further clarify the problem.

Study Reviewed

<u>Study</u>	<u>Results</u>	<u>Classification</u>
81-7. Acute delayed neurotoxicity-hens. Life Science Research #86/NKL039/551 July 7, 1987	Equivocal Results. "Axonal degeneration" in 5 of 12 diazinon treated hens but in 0 of 6 vehicle control hens. Histopathological lesions generally not clearly and unequivocally corroborated by behavioral changes. Dose level tested: 20 mg/kg.	SUPPLEMENTARY

Reviewed by: J.D. Doherty *12/14/87*
Section II, Tox. Branch (TS-769C)
Secondary reviewer: E.R. Budd *12/14/87*
Section II, Tox. Branch (TS-769C)

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DATA EVALUATION REPORT

STUDY TYPE: 81-7: Acute delayed neurotoxicity- TOX. CHEM. NO.: 342
hens.

ACCESSION NUMBER: 403311-01

MRID NO.: Not Provided

TEST MATERIAL: Stabilized technical grade Diazinon (96.2%, batch No. 86032). The sample was provided by Nippon Kayaku Co., Ltd., Japan. A copy of the specification sheet showing the contaminants of the sample is attached. See also discussion of the test material in the Appendix of this review.

SYNONYMS:

STUDY NUMBER(S): LSR Report No.: 86/NKL039/551

SPONSOR: Nippon Kayaku Co., Ltd., Japan.

TESTING FACILITY: Life Science Research, Ltd, Eye Suffolk IP23 7PX
England.

TITLE OF REPORT: Diazinon: Acute Delayed Neurotoxicity Study in the
Hen

AUTHOR(S): H.A. Cummins (Study Director), J.S.L. Fowler Ph.D. Pathologist

REPORT ISSUED: July 7, 1987 (Date report signed by authors).

CONCLUSIONS:

The results of this study are considered to be equivocal. "Axonal degeneration" was observed in 5 of 12 diazinon treated hens but in 0 of 6 control hens. Histopathological lesions, however, were generally not clearly and unequivocally corroborated by behavioral changes.

Classification: SUPPLEMENTARY Note: The design and execution of the study were consistent with current Guidelines but the equivocal result justifies the SUPPLEMENTARY classification.

Special Review Criteria (40 CFR 154.7): N/A (at this time).

Quality Assurance: A statement signed by D.J. Ford, Ph.D., Head of Quality Assurance Unit at LSR, attesting that 8 inspections were made was provided.

REVIEW

The objective of this study was to assess for any delayed type neurotoxicity potential of diazinon following acute oral administration to domestic hens (Sterling Ranger hybrid strain, Hill Farm, Suffolk, England).

1 Preliminary dose range finding study.

Three groups of 2 hens were dosed by gavage once with either 10, 20 or 40 mg/kg of the test sample of diazinon and observed for eight days. Both of the hens dosed with 40 mg/kg and a single hen dosed with 20 mg/kg died. From these data it was determined that 20 mg/kg was the approximate LD₅₀ for diazinon in hens and that this dose level would be used for the definitive neurotoxicity study. Note: no other data were provided regarding the range finding study. For example, the symptoms (degree, time of onset and duration) or day of death were not presented.

2. Definitive neurotoxicity study.

Following a seven week acclimation period, three groups of hens (about 12 months old weighting 1.89 to 2.70 kg) were segregated as follows:

<u>Group</u>	<u>Number of Hens</u>	<u>Treatment</u>
1	6	Maize Oil (Vehicle Control)
2	6	Positive Control (700 mg/kg tri-ortho-cresyl phosphate, TOCP in maize oil)
3	12	Diazinon (20 mg/kg in maize oil)

The test materials were given by gavage via a flexible rubber catheter introduced into the crop at a volume of 4 ml/kg bw. The hens were fasted overnight before administration of the test material and food was made available soon after dosing. After 21 days (on day 22) the above regimen of dosing was repeated and the hens were observed for an additional 21 days. All survivors were sacrificed on days 43-46.

The following is a discussion of the procedures used for examination and the results.

A. Initial signs of treatment (ChE inhibition effects).

[Note the hens treated with diazinon were also treated with atropine sulfate and pralidoxime to minimize the cholinesterase inhibition effects. The hens treated with maize oil and TOCP were not treated with atropine or pralidoxime.

Cholinergic signs resulting from inhibition of ChE enzymes were reported in all 12 of the hens dosed with diazinon but no such signs were reported in the hens dosed with maize oil or TOCP. The cholinergic signs included reduced activity, peripheral vasodilation (reddening of the comb and face), unsteadiness, drooped wings, resting on hocks and occasional salivation. These signs were controlled by atropinization and pralidoxime.

B. Behavioral signs of delayed neurotoxicity.

This aspect of the study consisted of a special test performed twice weekly which included exercising the hens by releasing the hens "as a flock into a floor pen". The hens were driven around the enclosed area and observed for inattentiveness, lethargy, or showing signs of deterioration of the locomotor system.

Two of the six hens in the vehicle control group showed some possible (but indefinite) symptoms. A single hen developed a "reddened comb and face" on day 9 but was otherwise normal. Another hen showed "reduced activity, unsteadiness and thin body conformation during days 26-28 and a single observation of reduced activity on day 34".

Three of the six hens in the TOCP (positive control) group showed definite signs of delayed neurotoxicity as observed by behavioral reactions. These birds showed "disturbed balance (unsteady on feet), unsteady gait and/or partial paralysis (inability to stand)" in four birds. Two of the birds were killed in extremis on day 16 and a third bird was killed in extremis on day 18. A fourth bird displayed signs of unsteady gait on days 13-17 and again on days 35-37 but did not develop more severe signs. The other two birds "showed no overt signs".

Table I of the study report (attached) summarizing the clinical signs of treatment indicates that no hens treated with diazinon showed "unequivocal signs of delayed neurotoxicity". However, some signs of possible delayed neurotoxicity were evident in the diazinon treated birds as indicated in the text of the results. These included a single bird (#572) developing gradual body weight loss and "few feces" from the beginning of the study. After the second dosing this bird was noted to have "reduced activity, unsteadiness, a swelling on its chest and resting on hocks". This bird was sacrificed in extremis on day 25. Because the symptoms following the second dosing were within a few days of dosing it is not possible to tell if these reactions were a consequence of delayed neurotoxicity. Four other birds displayed some evidence of a possible but indefinite indication of delayed neurotoxicity. For example, at least one bird (#573) was said to have "persistent reduced activity with unsteady stance following the second administration". This bird also showed a progressive body weight loss and perianal staining. The other three

birds (#'s 550, 556 and 568) were said to have transient episodes of reduced activity and/or unsteadiness of gait on days 35-39. One of these showed reduced activity from day 29 onwards during periods of forced exercise.

3. Histopathology

Following sacrifice (either scheduled or in extremis) via an overdose of pentobarbitone, the birds were perfused with 300-500 ml of 4% buffered formal saline (BFS). The brain and spinal cord and sciatic and tibial nerves were grossly examined and then excised after a 4 day fixation period of the carcass while being moistened with PFS. Transverse and longitudinal sections of the central and peripheral nerves were prepared. The tissue samples were dehydrated by immersion in a series of ascending concentrations of ethanol in water, embedded in paraffin and sectioned at a thickness of approximately 10 microns (peripheral nerves) and 10-15 microns (spinal cord and brain). The sections were mounted and stained with haematoxylin and eosin. Sections were also stained with Palmgren (axon specific) or Luxol fast blue (myelin specific). The following tissues were reported to be examined:

1. The medulla oblongata, cerebral and cerebellar cortex.
2. Upper cervical bulb of spinal cord (level 1).
3. Mid thoracic segment of spinal cord (level 2).
4. Lumbar-sacral segment of spinal cord (level 3).
5. Middle segment of sciatic nerve (longitudinal section approximately 20 mm).
6. Proximal region of the tibial nerve and its branches.

Only a single hen in the maize oil vehicle control group was reported to have any lesion of the nervous system. This was described as "focal gliosis" in the medulla oblongata and the degree was slight.

Four of the hens in the positive control group (TOCP treated) had lesions which were reported to unequivocally indicate a delayed type neurotoxic response. The study report considered the histopathological lesions in two other TOCP treated hens to be "equivocal with respect to indicating neurotoxicity (see Table 1, attached). These lesions consisted of axonal degeneration described as "minimal" or "slight" for a total of 16 incidences* for all nerve sections.

*Note: Table 3 of the study report reports a total of 11 incidences of "axonal degeneration" but compilation of the information from the individual animal pathology sheets indicates a total of 16 incidences among the TOCP treated hens.

The lesions in the nerve tissue of the TOCP treated hens were described as minimal or slight swelling and eosinophilic accumulations within axons. These were observed in the upper-cervical and/or mid-thoracic region of the spinal cord in all (6/6) TOCP treated hens. In addition, in some birds, similar observations were also made for sections from the lumbo sacral region of the spinal cord and/or the sciatic or tibial nerve. The tibial nerve had a lesion described as "perineural chronic inflammation" (slight) and "mucinous degeneration" (single incidence each of minimal and moderate). No histological changes were noted in the brain.

Five of the twelve hens in the diazinon treated group showed some histopathologic evidence of a possible delayed neurotoxic effect. For example, five hens developed "axonal degeneration" described as "minimal" and eosinophilic accumulations within the axons of the upper cervical and mid-thoracic regions of the spinal cord. The study report stated that these "lesions were generally less severe but qualitatively similar to those observed in the upper spinal cord of birds treated with tri-ortho-cresyl-phosphate" (refer to p. 22 of the report).

A single incidence of "focal necrosis" was reported in a hen which also had axonal degeneration. Two hens, both of which had axonal degeneration, also had "lymphocytic infiltration". No lesions in the brain were reported in the hens dosed with diazinon. As indicated previously, six hens treated with diazinon showed some possible evidence of behavioral changes. Of these six hens, however, only two also showed possible supporting evidence based on histopathological analysis. A summary TABLE showing both the behavioral and histopathological responses of each hen is attached.

4. Body Weight.

There was no definite difference among the body weights of the three groups of hens noted although some individual hens showed decreases in body weight gain.

CONCLUSION:

~~CORE~~ classification of this study is SUPPLEMENTARY. The study design and execution are considered to be consistent with the Guidelines; the equivocal result justifies the SUPPLEMENTARY classification.

The presence of "axonal degeneration" in five of the twelve hens treated with diazinon is disturbing, especially so because no similar lesion was found in the hens in the corn oil vehicle control group. Additional data will have to be provided to help establish whether diazinon did or did not cause delayed type neurotoxicity. A second delayed type neurotoxicity study with the same test material (Nippon Kayaku preparation) will have to be submitted to clarify the equivocal result.

TABLE: Summary of responses in hens treated with diazinon showing possible delayed neurotoxic response.

Hen #	Behavioral Response*	Histopathological Response	Both Effects
533	Yes	None	No
536	No	None	No
541	No	None	No
549	No	Axonal degeneration Lymphocytic infiltration	No
550	Yes	Axonal degeneration Lymphocytic infiltration	Yes
552	Yes	None	No
556	Yes	Axonal degeneration	Yes
566	No	None	No
568	Yes	None	No
571	No	Axonal degeneration	No
572	Yes	None	No
574	No	Axonal degeneration (2 levels) Focal necrosis	No

*Note the testing laboratory considers these responses to be equivocal and not necessarily evidence of a delayed type neurotoxic response.