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

JUN 7 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Isofenphos (Amaze), EPA Reg. #3125-326. Review of a New Acute Delayed Neurotoxicity Study in Hens (Study #83-418-01, Toxicology Report #453, 12/21/83) and Additional Information Concerning the Previously Submitted Neurotoxicity Studies. Acc.#252522 (and 252630*)
CASWELL#447AB

TO: William Miller, PM#16
Registration Division (TS-767)

FROM: Amal Mahfouz, Ph.D.
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Amal Mahfouz 6/4/84

THRU: Laurence D. Chitlik, DABT
Section Head, Toxicology Branch
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I.M. for L.D.C. 6/7/84

THRU: William L. Burnam, Chief
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Hazard Evaluation Division (TS-769)

Action Requested:

In response to the Agency request on 12/20/83 and 1/4/84 for additional neurotoxicity data, Mobay Chemical Corporation submitted for review the following data in Accession # 252522:

1. Report #86134. A new acute delayed neurotoxicity study (#83-418-01, Toxicology report #453 by E.J. Hixson, 12/21/83).

2. Report #34025*. This report included the raw data for a 1972 neurotoxicity study which was reviewed in 1978.

*NOTE: The raw data for the microscopic findings associated with the above mentioned 1972 study were later submitted on 3/5/84 in Accession #252630, Report #53881. These data will be also reviewed in this memo.

3. Report #80678. This report included a response to the Agency request for additional information concerning the neurotoxic esterase study (Toxicology report #255 by E.J. Hixson, 4/19/82) which was reviewed on 12/28/83. Pages 162, 166, 170, 174, 178, 182, 187, 188, 192, 198, 199, 205, and 206 of this report were illegible and were replaced by the registrant on 5/17/84.

4. Report #86248. A draft of a 1983 neurotoxicity study by B.W. Wilson of the University of California at Davis.

Recommendations:

1. The new acute delayed neurotoxicity study in hens (Report #86134, study #83-418-01, 12/21/83) indicated that Isofenphos did not cause a positive response at a dosage level of 32 mg/kg. The dosage was administered twice at a 21-day interval to hens protected with atropine and 2-PAM.

The study is classified as Core Minimum.

However, a higher dosage, 75 mg/kg, should have been tested in the above study instead of 32 mg/kg because the high level appeared to have a potential for positive response as noted in the 1983 study by B. Wilson. According to Wilson (Report #86248), Isofenphos appeared to cause delayed neurotoxicity in hens when administered orally at 75 mg/kg and when administered dermally at 100 mg/kg.

Thus, as previously requested by the Agency (letters to Mobay dated 12/20/83 and 1/4/84), a subchronic 90-day study in hens should be performed and submitted for review within a reasonable time.

2. Differences were noted between the raw data and the final reports in the 1972 studies on Oftanol acute delayed neurotoxicity (reports # 34025 and # 53881). These differences were associated with the number of animals tested, the date of testing, the reported clinical observation, and the duration of the observation periods, see report # 34025.

The raw data in report # 53881 also indicated that a potential for Oftanol to cause degenerative effects on the spinal cord was possible at 74 mg/kg. However, this effect could not be further determined in this dosage group because the histopathological examinations were not performed with myelin specific dye (luxol blue), see the discussion on pages 8 and 9 of this memo.

3. The attached letter of 2/9/84 by Mobay included a satisfactory response to most of the Agency's concerns relative to the previously submitted neurotoxic esterase (NTE) study, report #80678 (see the review of this study, dated 12/28/83). Hence, the study is upgraded from Invalid to Core Supplemental.

However, the raw data did not indicate the amount of the crude brain homogenate used in the determinations of NTE activity and the registrant should identify this amount. This issue is of concern to this reviewer because the method adopted in these determinations, Johnson's method (1977), had an error in the amount of the brain homogenate tested, i.e. the published value was 0.6 mg instead of 6.0 mg (personal communication with Johnson and other specialists).

4. The Agency already had access to Wilson's draft document, report #86248. This document was evaluated in my memos of 1/30/84, 12/28/83 and 12/7/83. A conclusion was made that although the protocol used by Wilson was primarily designed to investigate the effectiveness of antidotes against Oftanol in both chicken and rats, nevertheless his studies raised concerns about the potential for Oftanol to cause delayed neurotoxicity. In Wilson's studies, PAM and atropine protected chicken exhibited symptoms of delayed neurotoxicity when treated orally with 75 mg/kg Oftanol and dermally with 100 mg/kg.

Hence, the registrant was asked to perform a 90 day subchronic study in chicken, see also recommendation #1 above.

Review

1. Report #86134. Acute Delayed Neurotoxicity of Isofenphos (AMAZE). Toxicology Report #453 by E.J. Hixson, 12/21/83.

Accession No.: 252522

Sponsor: Mobay Chemical Corporation
Agricultural Chemicals Division
P.O. Box 4913, 8400 Hawthorn Road
Kansas City, Missouri 64120

Testing Facility: Mobay Chemical Corporation
Environmental Health Research
Corporate Toxicology Department
17745 South Metcalf
Stilwell, Kansas 66085

Study No.: 83-418-01

Dates: From 8/23 to 10/12/83
Report dated 12/21/83
Study submitted on 2/13/84

Study Personnel: E.J. Hixson (Study Director), H.E. Hoss (Pathology), P.D. Stewart (Conducted the Study), D.W. Lamb (Lab. Management), D.R. Mallicoat (Animal Care), and R.S. Schroeder (Analytical Chemistry and Quality Assurance).

Test Substance: Technical Isofenphos, 91.9% a.i., Batch #005281, a brown liquid supplied by Mobay Chemical Division. The test substance was stored in the freezer.

Vehicle: No vehicle was used, the test substance was administered undiluted.

Dosage: 32 mg/kg Isofenphos were orally administered to atropine and 2-PAM protected hens (group #1). An additional 32 mg/kg (with antidote protection) were administered to survivors after 21 days of the initial treatment. An untreated control (group #4) a negative control (group #2) and a positive (TOCP) control (group #3) were used.

All groups were observed for an additional 21 days after the second treatment of the surviving hens in group #1.

The following table reflects the number of animals in each of the above mentioned groups:

Group	1 Isofenphos Treated	2 Negative Control	3 Positive Control	4 Untreated Control
No. Animal	35[a]	5	10	5
Dosage[b] in mg/kg				
Isofenphos	32	-	-	-
Atropine[c]	50	50	-	-
2-PAM[c]	31	31	-	-
TOCP[d]	-	-	500	-

[a]Twenty hens were initially treated in this study. Fifteen hens were treated the following week and added to this group due to excessive mortality.

[b]For both the isofenphos group (#1) and the negative control group (#2) the initial treatments were repeated on day 21 after the initial exposure.

[c]Atropine was administered in group #1 after 30 minutes of the isofenphos administration; in both groups #1 and #2, 2-PAM administration followed atropine by 15 minutes.

[d]TOCP: tri-o-cresyl phosphate

Test Animals: Adult leghorn hens were used in this study. The birds were 12 months old and weighed an average of 1 kg (0.85 to 1.73 kg weight range) when initiated in this study.

Protocol: A copy of the protocol is attached to this review.

Results:

Isofenphos treated hens were compared to an untreated control group, a negative control (atropine + 2-PAM) group and a positive control (TOCP) group. The following results were noted in this study:

1. Mortality in the isofenphos group was 37% (13/35 birds died). Most of the deaths (9/13) occurred within the first two days of the initial treatment, a few (3/13) died within two days of the second dosage administration, and only one bird died after 7 days of the initial treatment. Hemorrhages, congested lungs or mesentery, gas filled intestines, and pale liver or spleen were common findings in the dead hens at necropsy. No death occurred in any other group in this study. No significant effect on body weights was observed in any group in this study.

2. Isofenphos did not cause any of the classical symptoms or histological lesions associated with delayed neurotoxicity when administered twice at 32 mg/kg dosage level to antidote protected hens. Each one of the two Isofenphos administrations was followed by a 21-day observation period.

When compared to the negative control group, only the positive control group reflected significant (alpha = 0.05) histopathological changes in the nervous system which were indicative of delayed neurotoxicity, i.e. demyelination, axonal degeneration, macrophage accumulation and degeneration digestive chambers were observed in the three sections of the spinal cord (cervical, thoracic and lumber). These lesions were moderate to severe.

No demyelination or axonal degeneration were observed in the isofenphos group. However, the incidence of hens with degeneration digestive chambers and macrophage accumulation in the lumber section of the spinal cord was significant (alpha = 0.05) when compared to the negative control group. These lesions were mild in this study.

(The Duncan's multiple range test was used for the above interpretation of the histopathological data).

3. TOCP treated hens exhibited symptoms of delayed neurotoxicity, i.e. after a few days of the initial treatment, several hens displayed reduced perching ability which remained until the end of the study.

The symptoms observed in the isofenphos group were not indicative of delayed neurotoxicity but were typical of those associated with acute cholinergic effects, i.e., salivation, ataxia followed by paralysis and death within the first 2 days of treatment. Some hens were unable to perch adequately during the first 3 days of treatment. However, complete recovery from all symptoms was observed in all survivors during the remainder of the study period.

4. The gross necropsy of the Isofenphos survivors was unremarkable from both the TOCP group and the negative control. Pale liver and fibrin tag on duodenum were occasionally observed in these 3 groups. No effect was observed in the untreated control group.

Discussion:

It appears that at the time this study was performed, a reseacher in California, Dr. Barry Wilson (University of California at Davis) had already indicated that isofenphos was suspected to cause delayed neurotoxicity in chicken at 75 mg/kg.

Thus, this reviewer questions the registrant's selection of a lower dosage, 32 mg/kg, for this test in view of the fact that Wilson's initial finding at this time did not show any effect at lower dosages and in view of the fact that a previous study by the registrant (#34025, 3/20/72) did not demonstrate any delayed neurotoxicity at 74 mg/kg or below.

It seems to me that a higher dosage, 75 mg/kg, should have been tested in this study in order to clearly verify both the registrant's previous negative finding at this level (study #34025/53881, 1972) and Wilson's recent positive findings (1983) at the same dosage level.

Conclusions:

In this study, isofenphos did not cause delayed neurotoxicity in hens at a dosage level of 32 mg/kg. The dosage was administered twice at a 21-day interval to hens protected with atropine and 2-PAM.

However, in the opinion of this reviewer, a higher dosage, 75 mg/kg, should have been tested in view of B. Wilson's recent findings (1983) concerning Isofenphos potential to cause delayed neurotoxicity at this dosage level and above.

Classification: Core Minimum.

2. Reports #34025 and 53881: Raw Data for a 1972 neurotoxicity study in hens, sponsored by Mobay (reviewed by EPA on 3/16/78 and reclassified as Core supplementary in 1983). Accessions #252522 and 252630.

The information recorded in the final report differed from the raw data as follows:

(a) All birds were observed for 21 days instead of 6 weeks.

(b) Seven birds were treated with TOCP instead of 5 birds, however, only 5 birds were histopathologically examined. None of these TOCP treated birds exhibited any symptoms during the 21 days osbervation period as noted in the raw data although the final report stated that those birds exhibited typical symptoms of delayed neurotoxicity from day 10 of treatment.

(c) In the atropine protected birds, 12 hens were treated with isofenphos at the 74 mg/kg dosage level as noted in the raw data instead of the recorded 10 birds in the final report; also, six birds died in this group instead of the reported 4 deaths. However, all six survivors were histopathologically examined as recorded in the final report.

Also, 9 atropine protected hens were treated with 100 mg/kg isofenphos instead of the recorded 10 birds in the final report, and six birds died in this group instead of the reported 7 deaths.

(d) No neurotoxic symptoms were recorded in the final report for birds treated with isofenphos. However the raw data indicated that several birds exhibited weakness of the leg immediately after treatment. This effect lasted for approximately five days in survivors of the LD_{50} dosage, 20 mg/kg for unprotected hens. In hens protected with atropine, the number of hens which exhibited this symptom and the duration of the symptom increased with the increase in the administered isofenphos dosage, i.e. at 74 mg/kg this symptom was observed for up to 13 days of the initial treatment.

Although the above symptom (weakness of leg) cannot be described as a typical delayed response because it occurred immediately after the compound administration, nevertheless the duration of this symptom at the higher dosages appeared to be of concern to this reviewer.

In summary, raw data for report #34025 (in-life clinical observations) reflected a poor reporting of the study findings. Often the number of dead animals and the number of animal tested at a specific dosage were not accurately reported, and apparently in some dosage groups the birds were not treated together on the same day.

In the raw data for report #53881, the histopathology request sheets indicated that hematoxylin and eosin were used as dyes in this study. However, the final report indicated that in the TOCP group (positive control), hematoxylin and eosin tissues which exhibited degenerative changes were further examined with luxol blue. According to the author these changes were not observed in other groups, which may indicate that these tissues were not further examined with luxol blue.

It is the opinion of this reviewer that the histopathological examination of the atropine protected hens treated with 74 mg/kg Isofenphos did not rule out the possibility of spinal cord degenerative activities in 3/6 birds examined: bird #29 had glial focus in the central grey matter, bird #31 had glial focus in the white matter, and bird #35 had a small perivascular focus of lymphoid cells. It is not clear if sections of the spinal cord in these 3 hens were further examined with luxol fast blue. Hence, this reviewer cannot adequately assess the potential degenerative effects of Oftanol on the spinal cord in this study.